DOCTORAL THESIS

Standardized ginger (Zingiber officinale) extract as a treatment for chemotherapy-induced nausea and vomiting: efficacy, safety and feasibility

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Standardized ginger (*Zingiber officinale*) extract as a treatment for chemotherapy-induced nausea and vomiting: efficacy, safety and feasibility

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Abstract

Despite significant advances in anti-emetic therapy, chemotherapy-induced nausea and vomiting (CINV) remains a significant burden to cancer patients. Ginger (*Zingiber officinale*) has shown promise as an adjuvant to standard anti-emetic therapy to allay CINV. It contains several bioactive compounds that could interact beneficially with the multiple pathways involved in this adverse outcome of treatment. However, the results of previous clinical trials testing ginger are equivocal and the extant literature has multiple limitations that require further investigation.

The primary purpose of this *Thesis by Publication* is to determine the efficacy, safety, and feasibility of ginger in clinical practice through a systematic program of literature reviews, and clinical, survey, and laboratory studies that account for the limitations of the extant literature, and current gaps in the knowledge.

The aim of the first study undertaken in this thesis was to investigate the potential mechanisms of action exerted by ginger on CINV. Certain active compounds in ginger act via antagonism of the 5-HT$_3$ receptors within the gastrointestinal tract leading to a possible reduction in CINV. Whether these compounds act directly at the serotonin binding site or act allosterically to modulate receptor activity has not been fully elucidated. Interactions between the principle compounds of ginger on the recently solved crystal structure of the murine 5-HT$_3$ receptor were investigated using *in silico* techniques, in order to characterise the sites and determine if a preference in binding affinity is evident within the two distinct binding sites (Chapter 6). The results of this study demonstrated the investigated ginger compounds exhibited high binding affinity at both sites. We postulated that these compounds may potentially act at both

sites – as seen with other serotonin modulators. The observed binding promiscuity of these compounds is likely due to their high degree of non-covalent interaction potential

The second study included in this thesis investigated the concentration of the primary bioactive compounds within 20 widely-available ginger products (including dietary supplements, beverages, and confectionary) using Reverse-Phase High-Performance Liquid Chromatography analysis (Chapter 7). This study addressed the efficacy and safety component of the projects aims by providing the following results. First, of the six dietary supplements analysed, standardized ginger extracts provided the most potent and consistent concentration of analysed ginger compounds, providing support for the use of standardized extracts in clinical trials. Second, when the concentration of compounds was presented by the approximate concentration that would be consumed in one serving, there were products from each product category that contained concentrations of the analysed compounds equal to, or exceeding, dietary supplements. This demonstrates that cancer patients could consume therapeutic concentrations of the active compounds within ginger through dietary intake alone. This has important implications for future clinical trials that aim to investigate the use of ginger supplementation. Furthermore, due to the potential effect ginger supplementation might exert on platelet aggregation, these results suggest that a high dietary intake of ginger products during chemotherapy could have safety implications. By analysing the concentration of primary compounds in a wide-range of commercially available ginger products, the information provided by this study will be able to inform Australian clinicians interested in these products for their adjuvant medicinal properties.

In the third and main study (Chapter 9), the efficacy and safety of ginger supplementation in humans was investigated in a clinical setting by way of a double-blind, randomized, placebo-controlled trial (N=51). This trial addressed the methodological limitations of the extant literature through the introduction of multiple robust features to the study design. These include following patients over an extended number of chemotherapy cycles, controlling for CINV-specific prognostic factors by recruiting only chemotherapy-naïve patients, implementing a dosing schedule consistent with the pharmacokinetics of oral ginger supplements, and independently analysing ginger supplements before and after the recruitment phase in order to ensure potency. The primary outcome was chemotherapy-induced nausea-related quality of life. Secondary outcomes included the severity, prevalence, and frequency of nausea, vomiting, and retching. This was also the first trial to assess the effect of ginger supplementation on cancer-related fatigue and nutritional status. The results of this study demonstrated a significant association between CINV- and nausea-related quality of life (p=0.043 and 0.029, respectively), global cancer-related quality of life (p=0.015), and cancer-related fatigue (p=0.007) in patients receiving the ginger intervention during the first cycle of chemotherapy. However, ginger supplementation did not reduce the prevalence or severity of CINV overall. There was no significant difference in reported adverse effects in the intervention group compared to the placebo group. By cycle 3 of chemotherapy, there was also significant attrition (33%). This suggests that the trial protocol could have been overly burdensome for participants and that the trial might not have been sufficiently powered to detect difference in CINV prevalence and severity. These results support previous studies, which indicate that ginger is well-tolerated; however, despite significant associations

between ginger supplementation and CINV-related quality of life (QoL), cancer-related QoL, and cancer-related fatigue, the use of ginger supplementation as an effective treatment for CINV is not supported by this trial.

The final study provided information regarding the feasibility of introducing dietary supplements such as ginger as a complement to routine clinical practice (Chapter 10). Healthcare professionals (N=370) responded to this survey, which assessed their current level of confidence, usage, and barriers with respect to recommending dietary supplements. The findings indicate mixed levels of confidence in recommending dietary supplements for their patients; nonetheless, there is strong interest in further training in this area despite the multiple barriers articulated, including concerns regarding drug-nutrient interactions.

In summary, the results of this thesis demonstrate that ginger supplementation is generally safe and feasible, and has several viable mechanisms of action related to CINV. While no reduction in the severity or prevalence of CINV were reported in our trial, ginger supplementation could be an effective and well-tolerated adjuvant intervention to enhance CINV-related QoL and reduce fatigue. Currently, healthcare professionals are interested in dietary supplements; however, further professional training in this area would improve the integration of dietary supplements into standard clinical practice. Future studies that explore the efficacy and the safety-profile of ginger are warranted in larger clinical trials.

Declaration

This *thesis by publication* is submitted to Bond University in fulfilment of the requirements of the degree of *Doctor of Philosophy*. This thesis represents my own original work towards this research degree and contains no material which has been previously submitted for a degree or diploma at this University or any other institution, except where due acknowledgement is made.

List of relevant publications and prizes

1.1 Relevant Peer-reviewed Publications

1. **Marx WM**, Teleni L, McCarthy AL, Vitetta L, McKavanagh D, Thomson D, et al. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. Nutrition Reviews. 2013;71(4):245-54. doi: 10.1111/nure.12016. Impact factor: 5.541 (5/76 Nutrition & Dietetics) Citations: 14 (Scopus)

2. **Marx W**, McCarthy A, Ried K, Vitetta L, McKavanagh D, Thomson D, et al. Can ginger ameliorate chemotherapy-induced nausea? Protocol of a randomized double blind, placebo-controlled trial. BMC Complementary and Alternative Medicine. 2014;14(1):134. PubMed PMID: doi:10.1186/1472-6882-14-134. Impact factor: 2.08 (6/24 Integrative and Complementary Medicine), Highly Accessed (>1000 views in first month). Citations: 2 (Scopus)

3. **Marx W**, Kiss N, Isenring L. Is ginger beneficial for nausea and vomiting? An update of the literature. Current Opinion in Supportive and Palliative Care. 2015;9(2):189-95. doi: 10.1097/spc.0000000000000135. PubMed PMID: 01263393-201506000-00018. Impact factor: 1.656 (47 of 89 Health Care Science & Service)

4. **Marx WM**, Ried K, McCarthy AL, Vitetta L, Sali A, McKavanagh D, et al. Ginger - mechanism of action in chemotherapy-induced nausea and vomiting: a review. (In Press). Crit Rev Food Sci Nutr. 2015. Impact factor: 5.176 (2/124

Food Science & Technology; 6/76 Nutrition & Dietetics). Citations: 2 (Scopus)

5. **Marx WM**, McKavanagh D, McCarthy AL, Bird R, Ried K, Chan A, Isenring E. The effect of ginger (*Zingiber officinale*) on platelet aggregation: a systematic literature review. PLOS One. *In Press* (7/10/2015). Impact Factor: 3.2

1.2 Publications submitted or in preparation

1. **Marx WM**, Kiss N, McCarthy AL, McKavanagh D, Isenring E. Chemotherapy-induced nausea and vomiting: a narrative review to inform dietetic practice. Journal of the Academy of Nutrition and Dietetics. Impact Factor: 3.467; Accepted 23/10/15.
2. **Marx WM,** McCarthy AL, Ried K, McKavanagh D, Frazer L, Vitetta L, Sali A, Lohning A, Schweiker S, Isenring E. The effect of a standardized ginger extract on chemotherapy-induced nausea and vomiting-related quality of life. BioMed Central Cancer. Impact factor: 3.36; Intended submission: December, 2015
3. **Marx WM,** Isenring E, Schweiker S, McCarthy AL, Ried K, Sali A, Vitetta L, Lohning A. The concentration of major active constituents within commercial ginger products using reverse phase-high performance liquid chromatography. Journal of Chromatography A. Impact factor: 4.298; Intended submission: December, 2015
4. **Marx WM**, Kiss N, McKavanagh D, Isenring E. The attitudes, beliefs and behaviours of healthcare professionals regarding dietary supplements.

BioMed Central Alternative and Complementary Medicines. Impact factor: 2.02; Intended submission: December, 2015

5. **Marx WM,** Isenring E, Lohning A. In silico investigation into the interaction between murine 5-HT3 receptor and principle compounds of ginger (Zingiber officinale). European Journal of Medicinal Chemistry. Impact factor: 3.447; Intended submission: December, 2015

1.3 Relevant Conference Abstracts and Presentations

1. **Marx WM**, Ried K, McCarthy AL, Vitetta L, McKavanagh D, Sali A, et al. Chemotherapy-induced nausea and vomiting: a review to inform clinical practice. Asia-Pacific journal of clinical oncology. 2013;9(S3):70. Impact Factor: 1.542 (174/211 Oncology)
2. **Marx WM**, Teleni L, McCarthy AL, Vittetta L, McKavanagh D, Thomson D, et al. Is ginger supplementation effective in ameliorating chemotherapy-induced nausea and vomiting? Asia Pacific Journal of Clinical Oncology; 2012: Wiley-Blackwell Publishing. Impact Factor: 1.542 (174/211 Oncology)
3. **Marx W**, Ried K, Mckavanagh D, Vitetta L, Sali A, Isenring L. Ginger as An Adjuvant for Chemotherapy-Induced Nausea and Vomiting: Where Does the Evidence Stand?, World Cancer Congress, 2014.
4. **Marx W.** Complementary and Alternative Medicines. *Invited lecturer*. Peter MacCallum Cancer Centre. 2014-2015
5. **Marx W.** Nutrition care in geriatric oncology. *Invited speaker*. Cancer Council Queensland. 2015

6. **Marx W**. Ginger for chemotherapy-induced nausea and vomiting. *Invited speaker*. Brisbane Diamantina Health Partners Cancer Collaborative Group Seminar Series. 2014

1.4 Relevant Prizes and Awards

1. Best Poster Prize, MASCC/ISOO Symposium. Miami, USA (July, 2014)
2. Best Poster Prize, Nutrition Society of Australia Conference, Australia (November, 2014)
3. Three time recipient of the Science of Nutrition in Medicine and Healthcare scholarship (2013-2015)

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To my incredible supervisory team and collaborators, thank you for the continued support throughout my thesis as well as the valuable feedback to my countless draft manuscripts.

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To A/Prof Anna Lohning, I cannot thank you enough for your incredible support and patience in guiding me through my laboratory work. Your input into my thesis has given me a richer perspective on this clinical issue and has allowed me to

pursue research questions that I would not have previously been confident to pursue. I have thoroughly enjoyed working with you during our blitz weeks and extended teleconferences. I cannot help but wonder though, what will we do with all our spare time now that our marathon teleconferences have finished?

I would also like to sincerely thank Prof Avni Sali, Prof Luis Vitetta, A/Prof Stephanie Schweiker, A/Prof Robert Bird, Dr Nicole Kiss, and Dan McKavanagh for your expertise, support, and input.

Thank you to my friends and family, particularly my parents whose unconditional love and support in everything that I have undertaken has given me the strength and confidence to pursue this project.

Finally, to my amazing fiancé, Thea, thank you. Thank you for your unwavering support, thank you for your patience and understanding, and thank you for everything that you do for me. You are my partner in crime, my driving inspiration, and my source of strength.

1 Table of Contents

List of figures

List of tables

List of abbreviations

Abbreviations included in this thesis are listed below.

5-HT3 – 5- Hydroxytryptamine

AA - Arachidonic acid

ADP - Adenosine diphosphate

CAM – Complementary and Alternative Medicine

CIN – Chemotherapy-induced Nausea

CINAHL - Cumulative Index to Nursing and Allied Health Literature

CINV – Chemotherapy-induced Nausea and Vomiting

CRF - Chemotherapy-Related Fatigue

CTZ – Chemotherapy Trigger Zone

ESAS - Edmonton Symptom Assessment System

FACIT-F - Functional Assessment of Cancer Therapy Fatigue

FACIT-F - Functional Assessment of Chronic Illness Therapies- Fatigue

FACT-G - Functional Assessment of Cancer Therapy- General

FLIE-5DR - The Functional Living Index – Emesis – 5 Day Recall

HEC, MEC, LEC – Highly/Moderately/Low Emetogenic Chemotherapy

HPLC – High Performance Liquid Chromatography

INR - International normalized ratio

INVR - Index of Nausea, Vomiting, and Retching

MANE – Morrow Assessment of Nausea and Emesis

MASCC - Multinational Association of Supportive Care in Cancer

MNT – Medical Nutrition Therapy

NCA - Non-competitive antagonist

NF-κB - Nuclear factor kappa-B

NHMRC – National Health and Medical Research Centre

NK1 - Neurokinin 1

PG-SGA – Patient Generated Subjective Global Assessment

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses

QoL – Quality of Life

RCT – Randomized Controlled Trial

RP-HPLC – Reverse Phase - High Performance Liquid Chromatography

TxB2 - Thromboxane B2

"*[The nausea] was just so consuming at times that I really couldn't think about anything else, and I just certainly couldn't, I just couldn't function. The only thing I could do was just curl up in bed*." - One patient's description of their experience with chemotherapy-induced nausea and vomiting[1]

Introduction

Two-thirds of chemotherapy patients report significant nausea,[2] which they often rate as the most disabling side-effect of their treatment.[3] Research consistently demonstrates that CINV deleteriously affects patient quality of life (QoL) and function, most significantly impairing their ability to undertake normal family and employment roles.[4] Persistent CINV can also result in malnutrition, cancer treatment delays and dose reductions, culminating in poorer treatment outcomes.[5,6] When nausea and vomiting are measured separately, nausea is reported to affect QoL to a greater extent than vomiting.[4,7] Hence, while interventions to reduce vomiting clearly benefit the patient, due to the high prevalence and severe impact of nausea on QoL, interventions that aim to treat chemotherapy-induced nausea should be prioritised.

Empirical data demonstrate that ginger root (*Zingiber officinale*) could be an effective anti-CINV agent, particularly with respect to nausea.[8] The bioactive compounds within ginger interact with several pathways that are implicated both directly and indirectly in CINV. These properties include 5-HT$_3$, substance *P* and acetylcholine receptor antagonism; as well as the modulation of cellular redox signalling, gastrointestinal motility, and gastric emptying rate.[9] Clinical trials also provide preliminary support, with several randomised-controlled trials reporting efficacy in the chemotherapy setting as well as in the context of gestational nausea, motion sickness, and post-operative nausea and vomiting.[8,10,11] However, due to the equivocal results and acknowledged limitations of these studies, further research is required before ginger can be recommended as a complement to standard antiemetic therapy for cancer patients.

There is also significant clinician and patient interest in evidence-based complementary therapies to prevent or relieve CINV. Cancer patients with persistent nausea often seek additional treatments to manage their symptoms, with up to 40% of cancer patients requesting additional information on supplements to help with side effects.[12] Studies indicate that ginger use by patients, and the recommendation of ginger as a supplement by clinicians, is also common in western countries. For example, a recent survey of UK oncologists found that 38% of respondents recommended ginger as a nausea treatment to their patients.[13] An additional concern is that patients do not consistently discuss their use of CAM with their physician, resulting in potential contraindications and safety concerns.[14]

Due to the need to improve current control of CINV, coupled with the rising use and interest in ginger as a therapeutic agent, the need to systematically evaluate its efficacy, safety, and feasibility in this setting is paramount. Therefore, the overall aim of this thesis was to answer the following research question:

What is the efficacy, safety, and feasibility of ginger as an adjuvant treatment for chemotherapy-induced nausea and vomiting in chemotherapy-naïve patients undergoing highly- and moderately- emetogenic chemotherapy?

1.5 Aims and Objectives

In order to address the research question, the following aims and outcomes were devised, with hypotheses generated for certain outcomes.

Aim: To determine the *efficacy* of ginger as an adjuvant treatment for CINV

Outcomes:

- To describe the mechanisms of action by which ginger could improve chemotherapy-induced nausea and vomiting.
- To determine the optimal form of ginger to be used as an adjuvant therapy in clinical trials.
- To determine the effect of ginger on i) CINV-related QoL and ii) the incidence, frequency and severity of chemotherapy-induced nausea and vomiting in chemotherapy-naïve patients receiving moderately or highly emetogenic chemotherapy regimens. The hypotheses associated with this aim are that, in chemotherapy-naïve cancer patients prescribed moderately or highly emetogenic therapy, compared to placebo:
    - H_1*:* The standardized ginger extract will provide a significant reduction in measures of CINV-related QoL in patients receiving moderately or highly emetogenic chemotherapy regimens compared to placebo.
    - H_2*:* The standardized ginger extract will provide a significant reduction in measures of acute chemotherapy-induced nausea in patients receiving moderately or highly emetogenic chemotherapy regimens compared to placebo.

Aim: To determine the *safety* of ginger as an adjuvant treatment for CINV

Outcomes:

- To determine the dosage of bioactive ginger compounds within a variety of ginger products.
- To assess the safety profile of ginger in a clinical setting, including adverse effects and contraindications.

Aim: To determine the *feasibility* of ginger as an adjuvant treatment for CINV

Outcomes:

- To determine the perceived confidence, reported use, and barriers for the use of dietary supplements such as ginger in clinical practice.
- To assess patient adherence to a standardized ginger regimen in a clinical setting.

1.6 Thesis Orientation

This Doctor of Philosophy research program is presented as a thesis by publication. Ten chapters of this thesis are manuscripts that have either been published in peer-reviewed journals (n=5) or are in various stages of the submission process (n=5). The thesis is separated into three parts. Part One comprises a series of published systematic and narrative literature reviews that detail the extant evidence regarding the role of dietetic management in CINV and the evidence regarding the clinical efficacy and safety of ginger supplementation during chemotherapy, as well as its suspected mechanisms of action. This includes the following chapters.

Chapter 1 is a narrative review accepted for publication in the *Journal of Academy of Nutrition and Dietetics* (2014 Impact Factor: 3.467). This chapter introduces the concept of chemotherapy-induced nausea and vomiting and discusses issues that relate to the dietetic management of these symptoms.

Chapter 2 is a systematic literature review of clinical data that assessed the evidence base for adjuvant ginger supplementation targeting chemotherapy-induced nausea and vomiting. This manuscript was published in *Nutrition Reviews* (2014 Impact Factor: 5.541; Scopus Citations: 14).

Chapter 3 complements Chapter 2 by reviewing the clinical trials conducted since the publication of the original systematic literature review. This was the result of an invitation by the editors of *Current Opinion in Supportive and Palliative Care* (2014 Impact Factor: 1.656) to provide an update on recent clinical data regarding the use of ginger for nausea from any stimuli (e.g. CINV as well as morning sickness, motion sickness).

Chapter 4 discusses the suggested mechanisms of action of ginger in relation to CINV and provides recommendations for future research. This manuscript was published in *Critical Reviews in Food Science and Nutrition* (2014 Impact Factor: 5.176; Scopus Citations: 2).

In Chapter 5, the potential effect of ginger on platelet aggregation, a widely-cited safety concern, was reviewed using existing clinical and observational data. This was recently published in *PLOS One* (2014 Impact Factor: 3.2).

Part Two comprises the four laboratory, clinical and survey studies undertaken during this PhD program. The first study aimed to investigate one of the mechanisms

of actions of ginger. While there are multiple potential pathways through which the bioactive compounds of ginger could ameliorate CINV, the exact mechanism is currently unknown (*see Chapters 3 and 4*). One promising hypothesis is that the primary compounds within ginger could interact with the 5-HT_3 receptors within the gastrointestinal tract, through a currently unknown binding site, in order to reduce CINV. In Chapter 7, through the use of *in silico* modelling techniques, this potential binding site was elucidated through investigation of the binding characteristics of the principle ginger compounds on two distinct areas of the murine 5-HT_3 receptor. This manuscript, currently in the advanced stages of preparation, will be submitted to *European Journal of Medicinal Chemistry* (Impact factor: 3.447)

In Chapter 8, the concentration of bioactive compounds within several commercial ginger products, including various dietary supplements, beverages, spices, and confectionery, was analysed in order to 1) determine the suitability of various types of ginger supplements as an adjuvant to standard pharmacological practice; 2) determine the amount of bioactive compound that can be consumed through dietary intake of ginger-containing food products; and 3) inform healthcare professionals interested in the medicinal use of ginger from an evidence-based perspective. This manuscript, also in the advanced stages of preparation, will be submitted to *Journal of Chromatography A* (Impact factor: 4.169).

The main study of this thesis is presented in Chapters 9 and 10. A double-blind, randomised, placebo-controlled trial was designed and implemented to rigorously investigate the efficacy, safety, and feasibility of a standardised form of adjuvant ginger supplementation for CINV. The protocol for this study was published

in *BioMed Central Complementary and Alternative Medicine* (Impact factor: 2.02; Scopus Citations: 2). The results of this study, which addressed the significant limitations apparent in the literature, has the potential to advance understanding regarding the viability of ginger in the oncology setting and will be submitted to *BioMed Central Cancer* (Impact factor: 3.36).

In order to ensure that dietary supplements can be utilised effectively during clinical practice, it is important to determine the current barriers, needs and behaviours of healthcare professionals regarding the use and recommendation of dietary supplements. In Chapter 11, a survey of 370 healthcare professionals determined their attitudes, beliefs and behaviours regarding dietary supplements. This manuscript will be submitted to *BioMed Central Complementary and Alternative Medicine* (Impact factor: 2.02) by the end of 2015.

Part Three is the final section of this thesis. In providing an overall discussion of the results obtained in Parts One and Two, it answers the research question driving this thesis; that is, how effective, safe and feasible is ginger supplementation in the clinical setting. This includes a discussion regarding the strengths and limitations of the studies undertaken in the course of my PhD candidature (Chapter 13), the overall implications of these studies, recommendations for clinical practice, and future research questions (Chapter 14).

Part One: Literature review

In order to determine the current state of the science and the knowledge gaps within the literature, a series of narrative and systematic literature reviews were undertaken. The aim of these reviews was to 1) provide an overview of the effect of CINV in the current oncology setting; 2) to determine the clinical efficacy and safety profile of ginger during chemotherapy; and 3) review relevant mechanisms of action. Please note the referencing styles included in the following chapters are in accordance with the respective journal guidelines.

Chapter 1. Chemotherapy-induced nausea and vomiting: a narrative review to inform dietetic practice.

This chapter introduces the concept of chemotherapy-induced nausea and vomiting and discusses issues that relate to the dietetic management of these symptoms. This chapter also briefly introduces the evidence-base for ginger supplementation for CINV; however, more detailed reviews of the literature are included in Chapter 2 and 3. Themes from this manuscript were presented at the following conference:

Wolfgang M Marx, Alexandra L McCarthy, Luis Vitetta, Dan McKavanagh, Damien Thomson, Avni Sali, Karin Ried, Elisabeth Isenring. Chemotherapy-induced nausea and vomiting: a guide for dietetic practice. *Dietitians Association of Australia 30th National Conference* (23– 25 May 2013, Sydney). Oral presentation.

Submission status: Accepted for publication in the *Journal of the Academy of Nutrition and Dietetics* (Impact factor: 3.467; Accepted 23/10/15)

1.1 Abstract

Chemotherapy-induced nausea and vomiting (CINV) are common nutrition-impact symptoms experienced by cancer patients. They exert a detrimental effect on dietary intake, risk of malnutrition and quality of life. While CINV are primarily managed with medication, dietitians play an important role in the management of CINV-related complications such as reduced dietary intake. This review discusses the burden of nausea and vomiting which cancer patients can experience, including its effect on quality of life, nutrition status, and treatment outcomes. Implications for dietetic practice include the need to explore the nature of reported symptoms, identify predisposing risk factors, and to consider the use of a variety of interventions that are individualised to the patient's symptoms. There are little clinical data regarding effective dietetic interventions for nausea and vomiting. In summary, this review discusses dietetic-related issues surrounding CINV including the pathophysiology, risk factors, prevalence, and both pharmacological and dietetic treatment options.

1.2 Introduction

There are multiple chemotherapy agents that can induce nausea and vomiting. However, with the advent of modern anti-emetics, there has been a significant reduction in the prevalence of vomiting, with a current estimated incidence of less than 20%.[1, 2] Efforts to control nausea in this setting have been less effective, with up to 60% of patients reporting nausea despite the use of anti-emetic medication.[1] Consequently, nausea remains one of the most distressing side effects experienced by cancer patients, while vomiting is now of less concern.[3-5] In addition, research has consistently associated chemotherapy-induced nausea and vomiting (CINV) with adverse effects on dietary intake, risk of malnutrition and quality of life (QoL).[6, 7]

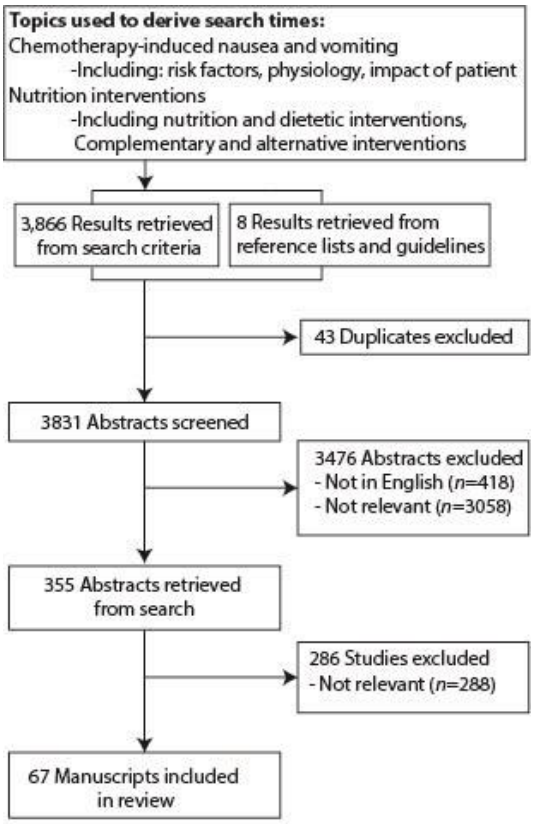
Dietitians routinely consult with cancer patients experiencing CINV and related symptoms. The aim of this manuscript is to inform dietetic practice by providing a general overview of CINV, as well as CINV-specific issues related to clinical nutrition. These include the pathophysiology, and management options for CINV, including current medications and potential dietetic treatment options.

1.3 Methods

A literature search was undertaken between January and July 2015 using the following databases: Medline, Cumulative Index to Nursing and Allied Health Literature, and the Cochrane Library. Search terms were not limited by timeframe; instead, all searches were from the date of each database's inception until July 2015.The bibliographies of relevant articles were scanned to identify additional articles of interest. The evidence-based guidelines of the Academy of Nutrition and Dietetics, Dietetics

Association of Australia and the Practice-based Evidence in Nutrition Knowledge Pathway were reviewed for additional references. The following search terms were used: (Chemotherapy AND (nausea OR vomiting OR CINV)) AND ((Risk factors OR prognostic OR predictor) OR (Mechanism OR pathophysiology OR physiopathology) OR (Nutrition OR malnutrition OR weight) OR "Quality of life" OR guidelines OR ginger OR protein OR (CAM OR Complementary OR Alternative)). Only studies published in English with human subjects were included. The results of this search strategy are detailed in Figure 1 and include the following citations:[1-67]. The results of the literature search were sorted based on the headings included in this review and were used to inform the discussion of each topic.

Figure 1-1 Flow diagram of literature search process conducted between January and July 2015



1.4 Defining chemotherapy-induced nausea and vomiting

CINV is a collective term used to describe the presentation of nausea, vomiting or a combination of both symptoms associated with the administration of cytotoxic chemotherapy. While nausea and vomiting are related concepts, they involve distinct physiological mechanisms and are therefore defined separately in Table 1-1.[68]

Nausea is a subjective sensation of discomfort, typically associated with the epigastrium, which might result in vomiting. Due to this subjective nature, the sensation, location, duration and intensity of nausea reported by patients can vary.[30] In addition, multiple nutrition impact symptoms interlink with nausea such as appetite loss, lack of

energy, taste changes and pain.[31] Hence, if a patient experiences nausea, it is prudent to investigate the individual's sensations in order to effectively target treatment towards those symptoms.

Table 1-1 Definitions of chemotherapy-induced nausea, vomiting and retching.

Symptom	Definition
Vomiting	Reflexive, rapid, and forceful oral expulsion of upper gastrointestinal tract contents due to powerful and sustained contractions in the abdominal and thoracic musculature.[70]
Nausea	Unpleasant, subjective feeling of discomfort, typically associated with the epigastrium that can result in vomiting. While nausea can cause pain and/or stress, it is considered as a distinct concept.[69]

CINV is further classified as *acute*, *delayed*, *anticipatory*, *breakthrough*, and *refractory*. Exact definitions of *acute CINV* vary but it is generally considered to be nausea and/or vomiting that occurs within 24 hours of chemotherapy administration.[32] *Delayed CINV* is defined as nausea and/or vomiting that occurs after the first 24 hours post-chemotherapy.[68] While this distinction might appear arbitrary, research suggests that differing physiological processes are involved in the acute phase when compared to the delayed phase.[69]

Anticipatory CINV is a conditioned response that occurs after previous cycles of chemotherapy in which nausea and/or vomiting were not adequately controlled. The current understanding of anticipatory CINV is explained in Pavlovian terms. According to this framework, a neutral stimulus (e.g. the smell of the hospital, the sight of treating staff) is coupled with an unconditioned response (CINV), caused by the unconditioned stimuli (chemotherapy). Once this occurs, a conditioned response develops wherein the formerly neutral stimulus elicits the same response as the unconditioned stimulus.[33] While a conditioning period is required for this coupling to occur, the length of this period varies according to the individual and can occur as soon as the second cycle of chemotherapy. Anticipatory CINV may also cause of certain food aversions, as food eaten during the days surrounding chemotherapy can be mentally paired with the sensation of nausea.

Breakthrough CINV is nausea and/or vomiting that occurs despite adherence to optimal anti-emetic protocols and is treated by administering additional "rescue" anti-emetic medication.[34] *Refractory CINV* comprises symptoms that occur in subsequent cycles despite delivery of optimal anti-emetic control in previous cycles.[34] If this occurs, additional medication is likely to be required.

1.5 Risk factors

An individual's risk of developing CINV is influenced by numerous factors (Table 2), which can be categorised into four broad categories: previous experience with nauseating stimuli (e.g. previous history of motion or morning sickness); genetic and trait factors (e.g. age and gender); psychosocial factors (e.g. anxiety); and finally, medical and

treatment-related factors (e.g. dose, type of chemotherapy). The primary determinant of a patient's risk of experiencing CINV is the emetogenic potential of the chemotherapy regimen. In order to guide anti-emetic therapy, chemotherapy regimens are stratified into the following classifications based on their emetogenic potential: minimally, fewer than 10% at risk; low , 10% to 30% of patients at risk; moderately, 30% to 90% of patients at risk; and highly emetogenic chemotherapy regimens, nearly all patients (> 90%) at risk.[34, 71]

Individual risk factors are associated with different levels of risk. For example, Molassiotis et al.[35] reported that patients with a history of nausea and vomiting (e.g. morning or motion sickness) were three times more likely to experience CINV (OR 3.2 , 95% CI: 1.29–7.95), while the odds of experiencing CINV increased by 69% for each incremental increase in reported pain (OR 1.69, 95% CI: 1.03–2.77). Patients with a greater number of these risk factors are more likely to experience CINV compared to patients with fewer traits. This has led to the development of multiple tools designed to predict the risk of CINV by assessing the cumulative effect of risk factors. For example, Bouganim et al.'s[36] tool to predict CINV risk demonstrated that patients categorized as at high-risk of CINV were three times more likely to experience symptoms than patients who were considered to be low risk. Predictive tools such as this are currently being refined and validated in larger populations, but with further studies these tools could improve symptom control by helping to identify high-risk patients before chemotherapy begins.

Table 1-2 Individual risk factors of CINV

Medical or treatment-related factors
• Dose and emetogenicity of chemotherapy regimen[37] • History of inner ear infections[38] • Feeling of 'warm or hot all over' or sweating during previous treatment cycles[39] • Feeling of generalised weakness during previous treatment cycles[39] • Lack of food consumption before chemotherapy session[40]
Psycho-social factors
• Anxiety (both trait and state) [41] • Negative expectations of chemotherapy[41]
Previous experience with nauseating stimuli
• History of poorly controlled chemotherapy[42] • History of motion sickness or morning sickness[25,26] • History of low alcohol intake[72]
Genetic and trait factors
• Female gender[3,25] • Younger age (< 40-65 years old) [2, 43] • Genetic polymorphisms related to 5-HT_3 metabolism[73, 74]

1.6 Pathophysiology

The development of CINV is complex; this section briefly describes the pathophysiology in CINV development.

The trigger site for CINV is thought to be within the gastrointestinal tract. Chemotherapy agents can directly interact with enterochromaffin cells located within the gastric epithelium, resulting in the release of the neurotransmitters serotonin and substance *P*.[75] The released neurotransmitters then interact with receptors located upon the vagus nerve, which subsequently transmits afferent signals to the chemoreceptor receptor zone (CTZ), a section of the brain within the area postrema, via the nucleus tractus solitarius. It is thought that modern 5-HT_3 antagonist medications (e.g. ondansetron) interact with the 5-HT_3 receptors involved in this process, which then

mitigates the degree of afferent vagal signalling. Another neurotransmitter, substance *P*, is also implicated in the generation of CINV primarily by binding to NK_1 receptors located centrally within the brain. Stimuli transmitted using these two neuropeptides, as well as stimuli from other neurotransmitters (e.g. dopamine, histamine) and other regions of the brain (e.g. the amygdala), are processed by the CTZ and vomiting centre, which then coordinate the relevant musculature to induce a nausea and/or vomiting response.[76]

An additional source of afferent signalling is suggested to be via direct interaction with the area postrema, as this part of the brain has a semi-permeable membrane that enables direct interaction with emetic stimuli within the blood or cerebrospinal fluid.

1.7 Impact on patient

1.7.1 *Nutrition status*

Malnutrition is both a serious and prevalent concern within the oncology setting.[44] Estimates vary but between 30-50% of the general oncology population experience malnutrition and has been reported to be as high as 88% in certain populations (i.e. head and neck cancer patients).[45-47] Malnutrition is considered an independent risk factor for mortality, increased length of stay, secondary infections, and healthcare costs.[44, 48, 49] Patients who experience CINV are particularly susceptible to malnutrition due to the direct effect of nausea and vomiting (e.g. the expulsion of food) or through behavioural factors (such as avoiding certain foods in an effort to prevent future bouts of CINV). Furthermore, vomiting can impede accurate nutrition diagnoses as it can reduce the validity of recorded dietary intake. Both nausea and vomiting are considered nutrition impact symptoms that can result in malnutrition.[50-53] Cross-sectional and prospective

studies investigating the effect of CINV on a patient's risk of malnutrition have reported a significant link.[7, 54]

For example, in a cross-sectional study of cancer patients undergoing chemotherapy (N=121), CINV was associated with malnutrition, as assessed using the Patient Generated-Subjective Global Assessment, demonstrating that the majority of patients with severe CINV were malnourished.[7] Similarly, in a prospective study including 104 chemotherapy patients, patients that experienced severe acute (mean: 5 vs 8; p=0.003) and delayed nausea (mean: 5.1 vs 8; p=0.017) were associated with higher PG-SGA scores compared to patients who experienced less severe or no nausea. .[54] However, the authors of this study noted that the anti-emetic regimens prescribed to patients within this study were not congruent with current guidelines. Therefore, while the observed prevalence might reflect typical clinical practice, the incidence and severity of CINV within this cohort could be higher than what might be observed if current anti-emetic recommendations were implemented.

When weight loss was measured instead of malnutrition, similar associations were identified. In a retrospective analysis of cachectic patients with pancreatic cancer (N=107), the absence of nausea and vomiting was an independent determinant of weight stabilisation (OR 6.5, 95% CI: 1.6-27.2; p=0.010).[29] Another study in a mixed oncology population (N=254) found that the prevalence of vomiting was higher in patients that experienced significant weight loss (>5% usual body weight) compared to patients that experienced minimal weight loss(32% vs 14%, respectively; p=0.005).[55]

In summary, while few studies have purposely investigated the association between CINV and malnutrition, the existing literature is consistent in its support of this association. In particular, these studies suggest that in patients who experience CINV, nutritional status should be actively monitored and managed in order to reduce the risk of malnutrition.

1.7.2 *Quality of life (QoL)*

QoL is poorer amongst patients who experience CINV, either during the acute or delayed phase, compared to patients without these symptoms.[27, 28] Highly emetogenic chemotherapy regimens are more likely to reduce QoL than moderately- or low emetogenic regimens. This detrimental effect on QoL is exacerbated with each additional day of CINV and is often compounded as treatment progresses, because patients who experience CINV in their initial cycle of chemotherapy are more likely to report poorer CINV-related QoL in subsequent cycles.[27, 56] This indicates that the burden of CINV might be cumulative and affects future chemotherapy cycles if not adequately controlled during the first cycle.[25, 77] When nausea and vomiting are measured separately, the adverse effect of nausea on QoL has been reported to be greater than the effect of vomiting, which is particularly pertinent as the prevalence of nausea is higher when compared to vomiting.[57] This difference in effect on QoL is likely due to current antiemetic therapy being predominantly effective for controlling vomiting as compared to nausea.

1.7.3 *Physical function*

Uncontrolled CINV can lead to a number of potentially serious physical conditions and CINV-related hospital admissions. Due to the loss of potassium, sodium, chloride and water resulting from frequent or severe vomiting, CINV might result in dehydration, electrolyte disturbances, and acid-base imbalances.[24] Another concern is the risk of aspiration pneumonia, a condition where vomitus enters the bronchial tree, resulting in pneumonitis. This can lead to further complications and in some cases is fatal.[24] In severe cases of vomiting, oesophageal tearing and related bleeding and pain can occur. Nutritional deficiencies are also a potential issue due to inadequate dietary intake of nutrients secondary to nausea and the inability to digest consumed food due to vomiting. These conditions can be further exacerbated by additional comorbidities.[58] Finally, during the 1980s, CINV-related treatment termination was reported to occur in patients;[23] however, it is likely that the prevalence of CINV-related treatment termination has been significantly reduced due to the improvement in anti-emetic medications.[22, 59]

1.8 Pharmacotherapy of CINV

Multiple medications prevent and relieve the distressing symptoms of CINV. International evidence-based guidelines, such as those developed by the Multinational Association for Supportive Care in Cancer and the National Comprehensive Cancer Network, suggest the ideal combination and timing of the available anti-emetics, according to the emetogenicity of the chemotherapy treatment.[34, 71] It is now common practice to include this standardised, combination approach to provide optimal control of CINV. While these medications are effective in reducing CINV, there is no single

medication that offers complete protection during highly or moderately emetogenic regimens and therefore, the medications discussed below are administered in combination.[34]

5-HT_3 antagonists such as ondansetron, granisetron and palonosetron are important components of modern anti-emetic therapy. 5-HT_3 antagonists work by binding to the 5-HT_3 receptors within the gastrointestinal tract, which consequentially blocks afferent emetic signalling to the CTZ within the brain. Corticosteroids such as dexamethasone are used for their incidental anti-emetic attributes and are commonly prescribed in combination with other anti-emetics.[34] The mechanism of action for this class of drug is poorly understood but suggested mechanisms include the modulation of the capillary permeability of the CTZ, anti-inflammatory effects within the gastrointestinal tract, and the release of endorphins.[21] A relatively new class of anti-emetic medication is NK_1 antagonists such as aprepitant and fosaprepitant. These medications are believed to act centrally within the CTZ by inhibiting the actions of the neuropeptide, substance *P*.[60] NK_1 antagonists are used in combination, usually with dexamethasone and a 5-HT_3 antagonist. They are most effective for moderate to highly emetogenic chemotherapy, especially where delayed CINV occurs. Until the introduction of 5-HT_3 antagonists, metoclopramide was one of the primary anti-emetic medications used to treat CINV. It has been suggested that metoclopramide, as with other dopamine antagonists such as phenothiazine and butyrophenone, primarily interacts with dopamine D2 receptors within the central nervous system, eliciting a prokinetic effect on the gut and therefore regulating gut mobility. However, due to the superiority of the new

generation of anti-emetic therapy and the incidence of extrapyramidal reactions with high-dose metoclopramide, anti-emetic guidelines only recommend metoclopramide for low emetogenic regimens and as a rescue anti-emetic in breakthrough emesis.[34, 71]

1.9 Dietetic and lifestyle interventions

1.9.1 *Dietetic-related interventions*

Dietitians regularly recommend a number of strategies to help patients manage their nausea and vomiting during chemotherapy. Broadly, these are categorised as strategies that involve modification to meal types and/or composition, behavioural strategies that target the way food is consumed, and lifestyle or environmental strategies (Table 3).[78-80] While many of these strategies appear intuitive, there are currently no clinical trials that have specifically investigated the efficacy of these strategies in reducing measures of CINV. Furthermore, while there are guidelines for the dietetic management of CINV,[80, 81] the lack of clinical trials means that these guidelines largely rely on expert opinion. However, medical nutrition therapy (MNT) is an intervention delivered by a dietitian that is tailored to the individual's need and circumstances and utilises the strategies outlined in table 3. Therefore, despite the lack of studies specifically investigating dietary interventions for CINV, studies investigating MNT as an intervention may provide some evidence for the use of these strategies in the management of CINV.[44, 82]

The oncology guidelines of the Academy of Nutrition and Dietetics state that there is currently strong evidence that MNT improves multiple treatment outcomes in patients undergoing chemotherapy, radiation or chemoradiotherapy in ambulatory or outpatient

and inpatient oncology settings.[82] However, when studies that have investigated the use of MNT in chemotherapy have been analysed separately from studies that have investigated MNT during radiotherapy, the evidence remains strong to suggest that MNT improves clinical and patient-centred outcomes (e.g. quality of life) in patients receiving radiotherapy but less so in patients receiving chemotherapy. Updated evidence-based practice guidelines endorsed by the Dietetic Association of Australia, state that evidence that MNT during chemotherapy results in similar improvements in clinical or patient-centred outcomes is currently insufficient.[44] The authors of these guidelines found that while dietary supplements or simple dietary interventions (e.g. provision of handouts detailing food high protein and energy or basic nutrition counselling) were able to improve nutritional outcomes such as dietary intake and weight status, they did not find an improvement in quality of life or survival.

Table 1-3 Common dietetic-related interventions

Meal modification strategies
• Avoiding overly spicy, fatty, and sweet foods • Flavouring cold or warm drinks and foods • Drink cold clear fluids between meals such as cordial, lemonade, dry ginger ale or fruit juice • Using well-tolerated foods with neutral odors • Avoid unpleasant food textures • Preference for dry foods such as toasts, crackers, and cereals
Behavioural strategies
• Eating slowly • Small and frequent meals • Avoid skipping meals • Eating before feeling hungry, since hunger can increase nausea • Avoid overeating
Lifestyle or environmental strategies
• Staying away from the kitchen during food preparation. • Eating in a pleasant, cool environment with fresh air • Avoid strong odours such as perfumes and cleaning products • Undertake activities that might distract from ones nausea (e.g. exercise, hobbies)

Interventions obtained from the following sources[78-80]

There is preliminary support for the use of MNT as part of CINV management. In a small study (N=35) of ambulatory cancer patients, nausea modestly improved after a two month multidisciplinary intervention involving a dietitian as well as a physical therapist, social worker, nurse, and a physician (no *p* value reported).[20] Furthermore, two

randomized controlled trials that investigated the use of dietary counselling or nutrition supplements in colorectal and head and neck cancer patients undergoing radiotherapy found that the severity and incidence of CINV was reduced within participants who received dietary counselling.[19, 61] While this was in a population undergoing radiotherapy, the pathways involved in the generation of nausea and vomiting are thought to be similar to CINV. These studies therefore provide preliminary support for the use of dietary counselling for these symptoms. Further studies are required to investigate the use of MNT during chemotherapy to manage CINV and assess the effect on clinical outcomes such as survival, length of stay and QoL.

There is limited evidence that CINV is associated with taste changes. One study found that patients who reported experiencing CINV also reported greater levels of taste changes and metallic taste.[18] The nature of this relationship has not been elucidated, so it is unclear if the use of MNT to manage taste changes may also provide relief to nausea and vomiting symptoms.

1.9.2 *Protein-rich meal consumption*

Preliminary clinical data suggest the consumption of a mixed meal, and in particular, a protein-rich meal, might improve nausea and vomiting symptoms from a variety of nauseating stimuli, including chemotherapy. For example, a prospective study (N=143) reported that patients who did not consume food before chemotherapy were 6.8 times more likely to experience CINV compared to patients who reported eating meals prior to chemotherapy.[57] Jednak et al.[62] examined this effect further in a clinical trial that

investigated the effect of different macronutrients on nausea during pregnancy. The results indicated that a protein-rich meal significantly reduced nausea symptoms compared to both equicaloric carbohydrate and fat meals, and non-caloric meals. Subsequently, Levine et al.[17] explored this in 28 cancer patients undergoing chemotherapy and reported that a combination of ginger and protein supplementation resulted in a significant reduction in CINV. This effect was more pronounced in the group receiving the highest dose of protein, which indicates that protein supplementation might have been primarily responsible for the reduction in CINV.

The exact mechanism for this is unclear but it has been observed that during exposure to nauseating stimuli, the electrical rhythm of the stomach becomes dysregulated.[17] The ingestion of a meal maintains the normal physiological rhythm of the stomach, which might in turn reduce symptoms of nausea and vomiting. The observed superiority of protein in reducing nausea symptoms is attributed to its effect on gastrin secretion, which is believed to normalise gastric activity.[16] However, while the current evidence is supportive, further studies that include larger sample sizes are required, particularly in the chemotherapy setting.

1.9.3 *Ginger supplementation*

In vitro and animal research indicate that compounds within ginger might exert several effects on pathways relevant to CINV. These include 5-HT_3 receptor antagonism and the modulation of gastrointestinal motility and gastric emptying rate.[14] In a recent systematic literature review, seven clinical trials were included that tested doses between

0.5-2g of ginger capsules.[15] The results provide equivocal evidence, with two studies reporting no effect,[13, 63] three finding some effect,[12, 64, 83] and two studies in favour but with caveats that reduce the real world application of these results.[10, 65] Our review also identified multiple limitations within the literature such as a lack of control for anticipatory nausea and prognostic factors that might influence individual CINV response, inconsistent use of standardized ginger formulations and validated questionnaires, and the use of potentially suboptimal dosing regimens. Hence, while some evidence supports ginger as an adjuvant anti-CINV therapy, existing limitations must be addressed before firm recommendations for its use can be made.

1.9.4 *Additional complementary therapies*

Several additional complementary therapies have demonstrated varying degrees of efficacy. These include yoga, progressive muscle relaxation, massage, aromatherapy, hypnosis, exercise, education programs, and acupuncture-point stimulation.[8, 9, 66, 67] However, while many of these therapies are likely to be low-cost and have minimal side effects, further trials are required to address limitations within the literature such as small sample sizes and inconsistent results.

1.10 Conclusion

In summary, CINV poses a significant burden to patients undergoing chemotherapy with the potential to result in further medical complications, reduce QoL, and increase the risk of malnutrition. While some evidence of a benefit from dietary intervention using MNT or protein rich meals exists further research is required.

1.11 References

1. Hsieh RK, Chan A, Kim HK, et al. Baseline patient characteristics, incidence of CINV, and physician perception of CINV incidence following moderately and highly emetogenic chemotherapy in Asia Pacific countries. *Support Care Cancer.* 2015;23:263-272.
2. Molassiotis A, Saunders MP, Valle J, et al. A prospective observational study of chemotherapy-related nausea and vomiting in routine practice in a UK cancer centre. *Support Care Cancer.* 2008;16:201-208.
3. Sun CC, Bodurka DC, Weaver CB, et al. Rankings and symptom assessments of side effects from chemotherapy: insights from experienced patients with ovarian cancer. *Support Care Cancer.* 2005;13:219-227.
4. Russo S, Cinausero M, Gerratana L, et al. Factors affecting patient's perception of anticancer treatments side-effects: an observational study. *Expert Opin Drug Saf.* 2014;13:139-150.
5. Kuchuk I, Bouganim N, Beusterien K, et al. Preference weights for chemotherapy side effects from the perspective of women with breast cancer. *Breast Cancer Res Treat,.* 2013;142:101-107.
6. Ballatori E, Roila F, Ruggeri B, et al. The impact of chemotherapy-induced nausea and vomiting on health-related quality of life. *Support Care Cancer.* 2007;15:179 - 185.

7. Davidson W, Teleni L, Muller J, et al. Malnutrition and chemotherapy-induced nausea and vomiting: implications for practice. *Oncol Nurs Forum.* 2012;39:E340 - 345.

8. Ezzo JM, Richardson MA, Vickers A, et al. Acupuncture-point stimulation for chemotherapy-induced nausea or vomiting. *Cochrane Database Syst Rev.* 2006:CD002285.

9. Richardson J, Smith JE, McCall G, Richardson A, Pilkington K, Kirsch I. Hypnosis for nausea and vomiting in cancer chemotherapy: a systematic review of the research evidence. *Eur J Cancer Care.* 2007;16:402-412.

10. Sontakke S, Thawani V, Naik MS. Ginger as an antiemetic in nausea and vomiting induced by chemotherapy: A randomized, cross-over, double blind study. *Indian J. Pharmacol.* 2003;35:32-36.

11. Ryan JL, Heckler CE, Roscoe JA, et al. Ginger (Zingiber officinale) reduces acute chemotherapy-induced nausea: a URCC CCOP study of 576 patients. *Support Care Cancer.* 2012;20:1479-1489.

12. Pillai AK, Sharma KK, Gupta YK, Bakhshi S. Anti-emetic effect of ginger powder versus placebo as an add-on therapy in children and young adults receiving high emetogenic chemotherapy. *Pediatr Blood Cancer.* 2011;56:234-238.

13. Zick SM, Ruffin MT, Lee J, et al. Phase II trial of encapsulated ginger as a treatment for chemotherapy-induced nausea and vomiting. *Support Care Cancer.* 2009;17:563-572.

14. Marx W, Ried K, McCarthy AL, et al. Ginger-Mechanism of Action in Chemotherapy-induced Nausea and Vomiting: A Review. *Crit Rev Food Sci Nutr.* 2015:0.

15. Marx WM, Teleni L, McCarthy AL, et al. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. *Nutr. Rev.* 2013;71:245-254.

16. Levine ME, Muth ER, Williamson MJ, Stern RM. Protein-predominant meals inhibit the development of gastric tachyarrhythmia, nausea and the symptoms of motion sickness. *Aliment Pharmacol Ther.* 2004;19:583-590.

17. Levine ME GM, Koch SY, Voss AC, Stern RM, Koch KL. Protein and ginger for the treatment of chemotherapy-induced delayed nausea. . *J Altern Complement Med,.* 2008;14:545-551.

18. Wickham RS, Rehwaldt M, Kefer C, et al. Taste changes experienced by patients receiving chemotherapy. *Oncol Nurs Forum.* 1999;26:697-706.

19. Ravasco P, Monteiro-Grillo I, Marques Vidal P, Camilo ME. Impact of nutrition on outcome: a prospective randomized controlled trial in patients with head and neck cancer undergoing radiotherapy. *Head Neck.* 2005;27:659-668.

20. Glare P, Jongs W, Zafiropoulos B. Establishing a cancer nutrition rehabilitation program (CNRP) for ambulatory patients attending an Australian cancer center. *Support Care Cancer.* 2011;19:445-454.

21. Herrstedt J, Aapro MS, Smyth JF, Del Favero A. Corticosteroids, dopamine antagonists and other drugs. *Support Care Cancer,.* 1998;6:204-214.

22. Van Laar ES, Desai JM, Jatoi A. Professional educational needs for chemotherapy-induced nausea and vomiting (CINV): multinational survey results from 2388 health care providers. *Support Care Cancer.* 2015;23:151-157.

23. Wilcox PM, Fetting JH, Nettesheim KM, Abeloff MD. Anticipatory vomiting in women receiving cyclophosphamide, methotrexate, and 5-FU (CMF) adjuvant chemotherapy for breast carcinoma. *Cancer Treat Rep.* 1982;66:1601-1604.

24. Lindley CM, Hirsch JD. Nausea and vomiting and cancer patients' quality of life: a discussion of Professor Selby's paper. *Br J Cancer Suppl.* 1992;19:S26-29.

25. Schwartzberg L, Szabo S, Gilmore J, et al. Likelihood of a subsequent chemotherapy-induced nausea and vomiting (CINV) event in patients receiving low, moderately or highly emetogenic chemotherapy (LEC/MEC/HEC). *Curr Med Res Opin,.* 2011;27:837-845.

26. Morrow GR, Roscoe JA, Hickok JT, et al. Initial control of chemotherapy-induced nausea and vomiting in patient quality of life. *Oncology (Williston Park).* 1998;12:32-37.

27. Cohen L, de Moor CA, Eisenberg P, Ming EE, Hu H. Chemotherapy-induced nausea and vomiting: incidence and impact on patient quality of life at community oncology settings. *Support Care Cancer.* 2007;15:497-503.

28. Lachaine J, Yelle L, Kaizer L, Dufour A, Hopkins S, Deuson R. Chemotherapy-induced emesis: quality of life and economic impact in the context of current practice in Canada. *Support Cancer Ther.* 2005;2:181-187.

29. Davidson W, Ash S, Capra S, Bauer J. Weight stabilisation is associated with improved survival duration and quality of life in unresectable pancreatic cancer. *Clin Nutr.* 2004;23:239-247.

30. Olver IN, Eliott JA, Koczwara B. A qualitative study investigating chemotherapy-induced nausea as a symptom cluster. *Support Care Cancer.* 2014;22:2749-2756.

31. Molassiotis A, Farrell C, Bourne K, Brearley SG, Pilling M. An exploratory study to clarify the cluster of symptoms predictive of chemotherapy-related nausea using random forest modeling. *J Pain Symptom Manage.* 2012;44:692-703.

32. Jordan K, Sippel C, Schmoll H-J. Guidelines for Antiemetic Treatment of Chemotherapy-Induced Nausea and Vomiting: Past, Present, and Future Recommendations. *Oncologist.* 2007;12:1143-1150.

33. Roscoe J, Morrow G, Aapro M, Molassiotis A, Olver I. Anticipatory nausea and vomiting. *Support Care Cancer,.* 2011;19:1533-1538.

34. Roila F, Herrstedt J, Aapro M, et al. Guideline update for MASCC and ESMO in the prevention of chemotherapy- and radiotherapy-induced nausea and vomiting: results of the Perugia consensus conference. *Ann Oncol.* 2010;21:v232-v243.

35. Molassiotis A, Stamataki Z, Kontopantelis E. Development and preliminary validation of a risk prediction model for chemotherapy-related nausea and vomiting. *Support Care Cancer.* 2013;21:2759-2767.

36. Bouganim N, Dranitsaris G, Hopkins S, et al. Prospective validation of risk prediction indexes for acute and delayed chemotherapy-induced nausea and vomiting. *Curr Oncol.* 2012;19:e414-421.

37. Hesketh P. Chemotherapy-induced nausea and vomiting. *N Engl J Med.* 2008;358:2482 - 2494.

38. Molassiotis A, Yam BM, Yung H, Chan FY, Mok TS. Pretreatment factors predicting the development of postchemotherapy nausea and vomiting in Chinese breast cancer patients. *Support Care Cancer.* 2002;10:139-145.

39. Morrow GR. Clinical characteristics associated with the development of anticipatory nausea and vomiting in cancer patients undergoing chemotherapy treatment. *J Clin Oncol.* 1984;2:1170-1176.

40. Booth CM, Clemons M, Dranitsaris G, et al. Chemotherapy-induced nausea and vomiting in breast cancer patients: a prospective observational study. *J Support Oncol.* 2007;5:374-380.

41. Hickok JT, Roscoe JA, Morrow GR. The Role of Patients' Expectations in the Development of Anticipatory Nausea Related to Chemotherapy for Cancer. *J Pain Symptom Manage.* 2001;22:843-850.

42. Persistence of efficacy of three antiemetic regimens and prognostic factors in patients undergoing moderately emetogenic chemotherapy. Italian Group for Antiemetic Research. *J Clin Oncol.* 1995;13:2417-2426.

43. Pirri C, Katris P, Trotter J, Bayliss E, Bennett R, Drummond P. Risk factors at pretreatment predicting treatment-induced nausea and vomiting in Australian cancer patients: a prospective, longitudinal, observational study. *Support Care Cancer*. 2011;19:1549-1563.

44. Isenring E, Zabel R, Bannister M, et al. Updated evidence-based practice guidelines for the nutritional management of patients receiving radiation therapy and/or chemotherapy. *Nutr Diet.* 2013;70:312-324.

45. Segura A, Pardo J, Jara C, et al. An epidemiological evaluation of the prevalence of malnutrition in Spanish patients with locally advanced or metastatic cancer. *Clin Nutr*. 2005;24:801-814.

46. Unsal D, Mentes B, Akmansu M, Uner A, Oguz M, Pak Y. Evaluation of nutritional status in cancer patients receiving radiotherapy: a prospective study. *Am J Clin Oncol.* 2006;29:183-188.

47. Read JA, Choy ST, Beale PJ, Clarke SJ. Evaluation of nutritional and inflammatory status of advanced colorectal cancer patients and its correlation with survival. *Nutr Cancer.* 2006;55:78-85.

48. Pressoir M, Desne S, Berchery D, et al. Prevalence, risk factors and clinical implications of malnutrition in French Comprehensive Cancer Centres. *Br J Cancer.* 2010;102:966-971.

49. Pirlich M, Schutz T, Norman K, et al. The German hospital malnutrition study. *Clin Nutr*. 2006;25:563-572.

50. Isenring E, Bauer J, Capra S. The scored Patient-generated Subjective Global Assessment (PG-SGA) and its association with quality of life in ambulatory patients receiving radiotherapy. *Eur J Clin Nutr.* 2003;57:305-309.

51. Isenring E, Cross G, Daniels L, Kellett E, Koczwara B. Validity of the malnutrition screening tool as an effective predictor of nutritional risk in oncology outpatients receiving chemotherapy. *Support Care Cancer.* 2006;14:1152-1156.

52. Tong HT, Isenring EA, Yates P. The prevalence of nutrition impact symptoms and their relationship to quality of life and clinical outcomes in medical oncology patients. *Support Care Cancer,.* 2008;17:83-90.

53. Thoresen L, Fjeldstad I, Krogstad K, Kaasa S, Falkmer UG. Nutritional status of patients with advanced cancer: the value of using the subjective global assessment of nutritional status as a screening tool. *Palliat Med,.* 2002;16:33-42.

54. Farrell C, Brearley SG, Pilling M, Molassiotis A. The impact of chemotherapy-related nausea on patients' nutritional status, psychological distress and quality of life. *Support Care Cancer.* 2013;21:59-66.

55. Grosvenor M, Bulcavage L, Chlebowski RT. Symptoms potentially influencing weight loss in a cancer population. Correlations with primary site, nutritional status, and chemotherapy administration. *Cancer.* 1989;63:330-334.

56. Fernandez-Ortega P, Caloto MT, Chirveches E, et al. Chemotherapy-induced nausea and vomiting in clinical practice: impact on patients' quality of life. *Support Care Cancer.* 2012;20:3141-3148.

57. Bloechl-Daum B, Deuson RR, Mavros P, Hansen M, Herrstedt J. Delayed nausea and vomiting continue to reduce patients' quality of life after highly and moderately emetogenic chemotherapy despite antiemetic treatment. *J Clin Oncol.* 2006;24:4472-4478.

58. Bender CM, McDaniel RW, Murphy-Ende K, et al. Chemotherapy-induced nausea and vomiting. *Clin J Oncol Nurs.* 2002;6:94-102.

59. Neymark N, Crott R. Impact of emesis on clinical and economic outcomes of cancer therapy with highly emetogenic chemotherapy regimens: a retrospective analysis of three clinical trials. *Support Care Cancer.* 2005;13:812-818.

60. Hawkins R, Grunberg S. Chemotherapy-induced nausea and vomiting: challenges and opportunities for improved patient outcomes. *Clin J Oncol Nurs.* 2009;13:54-64.

61. Ravasco P, Monteiro-Grillo I, Vidal PM, Camilo ME. Dietary counseling improves patient outcomes: a prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy. *J Clin Oncol.* 2005;23:1431-1438.

62. Jednak MA, Shadigian EM, Kim MS, et al. Protein meals reduce nausea and gastric slow wave dysrhythmic activity in first trimester pregnancy. *Am J Physiol.* 1999;277:G855-861.

63. Fahimi F, Khodadad K, Amini S, Naghibi F, Salamzadeh J, Baniasadi S. Evaluating the Effect of Zingiber Officinalis on Nausea and Vomiting in Patients Receiving Cisplatin Based Regimens. *Iran J Pharm Res.* 2011;10:379-384.

64. Panahi Y, Saadat A, Sahebkar A, Hashemian F, Taghikhani M, Abolhasani E. Effect of ginger on acute and delayed chemotherapy-induced nausea and vomiting: a pilot, randomized, open-label clinical trial. *Integr Cancer Ther.* 2012;11:204-211.

65. Manusirivithaya S, Sripramote M, Tangjitgamol S, et al. Antiemetic effect of ginger in gynecologic oncology patients receiving cisplatin. *Int J Gynecol Cancer.* 2004;14:1063-1069.

66. Mustian KM, Devine K, Ryan JL, et al. Treatment of Nausea and Vomiting During Chemotherapy. *US Oncol Hematol.* 2011;7:91-97.

67. Raghavendra RM, Nagarathna R, Nagendra HR, et al. Effects of an integrated yoga programme on chemotherapy-induced nausea and emesis in breast cancer patients. *Eur J Cancer Care.* 2007;16:462-474.

68. Hesketh PJ. Management of Nausea and Vomiting in Cancer Treatment: Introduction, Scope of the Problem. In: Hesketh PJ, ed. *Management of Nausea and Vomiting in Cancer and Cancer Treatment*. Sudbury, MA: Jones and Bartlett; 2005:1-15.

69. Horn CC. Why is the neurobiology of nausea and vomiting so important? *Appetite.* 2008;50:430-434.

70. Wilhelm SM, Dehoorne-Smith ML, Kale-Pradhan PB. Prevention of Postoperative Nausea and Vomiting. *Ann Pharmacother.* 2007;41:68-78.

71. National Comprehensive Cancer Network (NCC). NCCN Practice Guidelines in Oncology™ [v.1.2015]: Antiemesis.: National Comprehensive Cancer Network; 2015.

72. Rubenstein EB. The Role of Prognostic Factors in Chemotherapy-Induced Nausea and Vomiting. In: Hesketh PJ, ed. *Management of Nausea and Vomiting in Cancer and Cancer Treatment*. Sudbury, MA: Jones and Bartlett; 2005:87-98.

73. Kaiser R, Sezer O, Papies A, et al. Patient-tailored antiemetic treatment with 5-hydroxytryptamine type 3 receptor antagonists according to cytochrome P-450 2D6 genotypes. *J Clin Oncol.* 2002;20:2805-2811.

74. Tremblay PB, Kaiser R, Sezer O, et al. Variations in the 5-hydroxytryptamine type 3B receptor gene as predictors of the efficacy of antiemetic treatment in cancer patients. *J Clin Oncol.* 2003;21:2147-2155.

75. Torii Y, Saito H, Matsuki N. Induction of emesis in Suncus murinus by pyrogallol, a generator of free radicals. *Br J Pharmacol Chemother.* 1994;111:431-434.

76. Rudd JA, Andrews, P.L.R. Mechanisms of acute, delayed, and anticipatory emesis induced by anticancer therapies. In: Hesketh PJ, ed. *Management of Nausea and Vomiting in Cancer and Cancer Treatment*. Sudbury, MA: Jones and Bartlett; 2005:15-65.

77. Morrow G, Roscoe J, Hickok J, et al. Initial control of chemotherapy-induced nausea and vomiting in patient quality of life. *Oncology (Williston Park).* 1998;12:32 - 37.

78. Escott-Stump S. *Nutrition and Diagnosis-related Care*: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.

79. Mahan LK, Raymond JL, Escott-Stump S. *Krause's Food & the Nutrition Care Process*: Elsevier Health Sciences; 2013.

80. Dietitians of Canada. Cancer - Nutritional Implications of Treatment: Key Practice Points. *Practice-based Evidence in Nutrition [PEN]* 2008.

81. American Cancer Society. Nutrition for the Person With Cancer During Treatment: A Guide for Patients and Families American Cancer Society; 2015.

82. Academy of Nutrition and Dietetics Evidence Analysis Library. Oncology (ONC) Guideline Academy of Nutrition and Dietetics,; 2013.

83. Ryan JL, Heckler CE, Roscoe JA, et al. Ginger (Zingiber officinale) reduces acute chemotherapy-induced nausea: a URCC CCOP study of 576 patients. *Support Care Cancer.* 2011.

Chapter 2. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review.

In this chapter, a systematic literature review of the clinical data was conducted to determine the evidence base for the use of adjuvant ginger supplementation for chemotherapy-induced nausea and vomiting. This manuscript was published in *Nutrition Reviews* (2014 Impact Factor: 5.541) and has been cited 14 times (07/08/2015; Scopus). Information from this manuscript was presented at the following conferences:

1. **Marx W**, McCarthy AL, Vitetta L, McKavanagh D, Thomson D, Sali A, Ried K, Isenring E. Is ginger effective in ameliorating chemotherapy-induced nausea and vomiting? Science of Nutrition in Medicine and Healthcare (3 – 5 May 2013, Sydney).

   Oral and poster presentation. Awarded Science of Nutrition in Medicine and Healthcare student scholarship
2. **Marx W**, McCarthy AL, Vitetta L, McKavanagh D, Thomson D, Sali A, Ried K, Isenring E. Ginger as an adjuvant for chemotherapy-induced nausea and vomiting: Where does the evidence stand? World Cancer Congress (3 – 6 December 2014, Melbourne).

   Oral presentation

Citation

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2.1 Abstract

Chemotherapy-induced nausea and vomiting (CINV) is a common side-effect of cytotoxic treatment. It continues to affect a significant proportion of patients despite the widespread use of anti-emetic medication. In traditional medicine, ginger (*Zingiber officinale*) has been used to prevent and treat nausea in many cultures for thousands of years. However, its use has not been confirmed in the chemotherapy context. To determine the potential use of ginger as a prophylactic or treatment of CINV, a systematic literature review was conducted. Reviewed studies comprised randomised controlled trials or cross-over trials that investigated the anti-CINV effect of ginger as the sole independent variable in chemotherapy patients. Seven studies met the inclusion criteria. All studies were assessed on methodological quality and their limitations were identified. Studies were mixed in their support of ginger as an anti-CINV treatment in patients receiving chemotherapy, with three demonstrating a positive effect, two in favour but with caveats and two showing no effect on measures of CINV. Future studies are required to address the limitations identified before clinical use can be recommended.

Key words: nausea, ginger, chemotherapy, CINV

2.2 Introduction

Chemotherapy is one of medicine's key interventions in the treatment of cancer. While cytotoxic interventions for cancer are efficacious, they are often accompanied by a variety of adverse effects. Chemotherapy-induced nausea and vomiting (CINV) is a relatively common side effect of this treatment. A combination of different classes of anti-emetic medications such as 5-HT_3 antagonists, neurokinin 1 (NK_1) receptor antagonists, corticosteroids and anti-anxiolytics have been shown to have additive effects and are commonly prescribed for patients having chemotherapy. Vomiting has now been largely controlled but efforts to control nausea have been less successful; affecting upwards of 60% of patients.[1] Persistent nausea is also considered the most distressing symptom for patients in this setting.[2,3] This is of particular concern in oncology patients as nausea and vomiting can adversely affect food intake, increasing the risk of malnutrition during treatment. Previous studies report one in two patients in this setting as malnourished.[4] The cumulative effect of pre-treatment and treatment-related malnutrition can be one of compromised immune function, decreased performance status, poor response to treatment, and sometimes, treatment discontinuation.[5-7]

The use of integrative or complementary therapies has been steadily increasing in western countries.[8] This wide-spread use of integrative therapies has resulted in an increased interest in the investigation of these therapies as either stand-alone or adjuvant treatments for treating clinical conditions. Ginger (*Zingiber officinale*) has a long history in many cultures as a folk-remedy for nausea and gastrointestinal discomfort. Empirical

research has demonstrated that ginger could be effective as an anti-nausea agent and in particular, it has been proposed as a possible candidate for anti-CINV therapy.

While the exact mechanism of action is unknown, multiple active constituents within ginger (i.e. gingerols, shogoals, zingiberene, zingerone, and paradol) have been identified as potentially exerting beneficial effects on multiple areas implicated in the pathophysiology of CINV. Cell culture and animal studies suggest that these constituents stimulate oral and gastric secretions, regulate gastrointestinal motility,[9,10] interact with the 5-HT_3 receptors implicated in the CINV reflex,[11] and assists in rescuing intracellular redox.[12,13] Furthermore, animal studies provide preliminary support for the role of ginger supplementation in the prevention of cisplatin-induced emesis.[14,15]

Few adverse effects from the ingestion of ginger are reported in the literature.[16] Oral ginger is generally well tolerated, with mild gastrointestinal adverse effects including abdominal discomfort, heartburn, and diarrhoea being the most commonly reported. Theoretically, ginger inhibits platelet aggregation which could result in excessive bleeding, however this has not been reported in practice.[17] When added to conventional anti-emetics used in the prophylaxis and treatment of CINV, ginger does not appear to increase adverse effects.[18] Indeed, conventional anti-emetics appear to have a more varied adverse effect profile (including more severe adverse effects) compared to ginger. For example, steroids such as dexamethasone used for short durations commonly cause gastrointestinal adverse effects such as dyspepsia and psychological effects such as

insomnia, while 5-HT_3 receptor antagonists such as ondansetron commonly cause constipation and headache.[19,20]

Whilst direct cost comparison between ginger and standard anti-emetic therapies is difficult due to lack of dose equivalency, it is likely that ginger would compare well, given its low ingredient cost and accessibility. Ginger is already readily available in several commercial non-prescription formulations, and requires little technical innovation in terms of cultivation and preparation.[21]

Ernst et al.[16] published a review on the effect of ginger on nausea and vomiting in a variety of settings, including only one paper that specifically investigated its effects on CINV. The review found that ginger was generally beneficial; however, firm conclusions could not be made due to the low number of studies in each setting. Multiple papers have since been published in this area and therefore, our review aims to detail the current published research from randomised, controlled trials (RCTs) and evaluate the efficacy of ginger in the prevention of CINV, highlighting areas for future investigation.

2.3 Method

A systematic search of the literature was conducted using PubMed, the Cochrane Library, and CINAHL, as well as bibliographies of past research on the subject (see Figure 2-1). Search terms were not limited by timeframe and therefore all searches were between April 2012 and the date of the databases inception. Articles were identified using the search terms "("*Zingiber officinale*" OR "ginger") AND ("cancer" or "chemotherapy") AND ("nausea" OR "emesis" OR "vomit" OR "CINV")". Inclusion criteria for this review were: 1) RCT and/or cross-over trials that used either placebo or

current anti-CINV treatment as a control; 2) In human participants, undergoing chemotherapy; 3) The use of ginger as the main intervention and specifically investigating its effects on nausea and vomiting; and 4) Published in English.

All studies included in this review were analysed for common characteristics and methodologies, major findings, and potential limitations. Additionally, all studies were individually rated for evidence level using the National Health and Medical Research Council (NHMRC) Hierarchy of Evidence guidelines (IV-I, with I being the strongest level of evidence) as well as assessed in terms of quality (positive, neutral, negative) using the American Dietetic Association's quality criteria checklist. [22,23]

The overall body of evidence (based on a summary of the individual studies) evaluated within this review was assessed using a separate tool, the NHMRC's body of clinical evidence assessment matrix, an assessment tool that assigns a letter grade (A: strongest to D: weakest) based on the strength of the literature included in a review. [22]

2.4 Results

The search strategy identified seven studies (Table 2-1) that provided Level II evidence and all had a positive quality rating. Hence, all studies included in this review possessed attributes consistent with rigorous scientific method, such as randomised group allocation and clear inclusion and/or exclusion criteria. Of note, two studies did not meet the inclusion criteria as they were unpublished literature (Pecoraro et al.[24], Pace et al.[25]) and two studies (Levine et al.[26], Meyer at al.[27]) were excluded as they utilised an ineligible study design.

2.4.1 *Study characteristics*

All seven studies included in this review were RCTs, three of which were cross-over trials. Two cross-over trials used current anti-CINV treatment as the control group rather than placebo.[28,29] Five of the seven studies had relatively small sample sizes (approximately 30-70 participants in total). Zick et al.[18] and Ryan et al.[30] were the exceptions, with 129 and 576 participants completing each trial respectively. The length and timeframe of symptom assessment varied between studies, with assessment of CINV symptoms conducted anytime from three days prior to chemotherapy treatment and up to 10 days post-treatment. The outcomes measured in the majority of studies (5/7) were acute nausea and vomiting (24 hours post-chemotherapy) and delayed nausea and vomiting (between two and ten days post-chemotherapy); however, Ryan et al.[30] did not measure vomiting symptoms and Sontakke et al.[28] measured acute nausea and emetic events only.

Typical dosing regimens were 1g to 2g of ginger, divided into four to eight capsules and consumed over a period of one to ten days. The majority of studies used powdered ginger preparations, while two studies used extracts that were standardised to either their gingerol content or to a combination of active compounds (shogoals, gingerols and zingerone). Zick et al.[18] independently verified the preparations using high-performance liquid chromatography to ensure the potency of the intervention and found their extract contained "5.38 mg (2.15%) 6-gingerol, 1.80 mg (0.72%) 8-gingerol, 4.19 mg (1.78%) 10-gingerol, and 0.92 mg (0.37%) 6-shogaol". Ryan et al.[30] reported that the

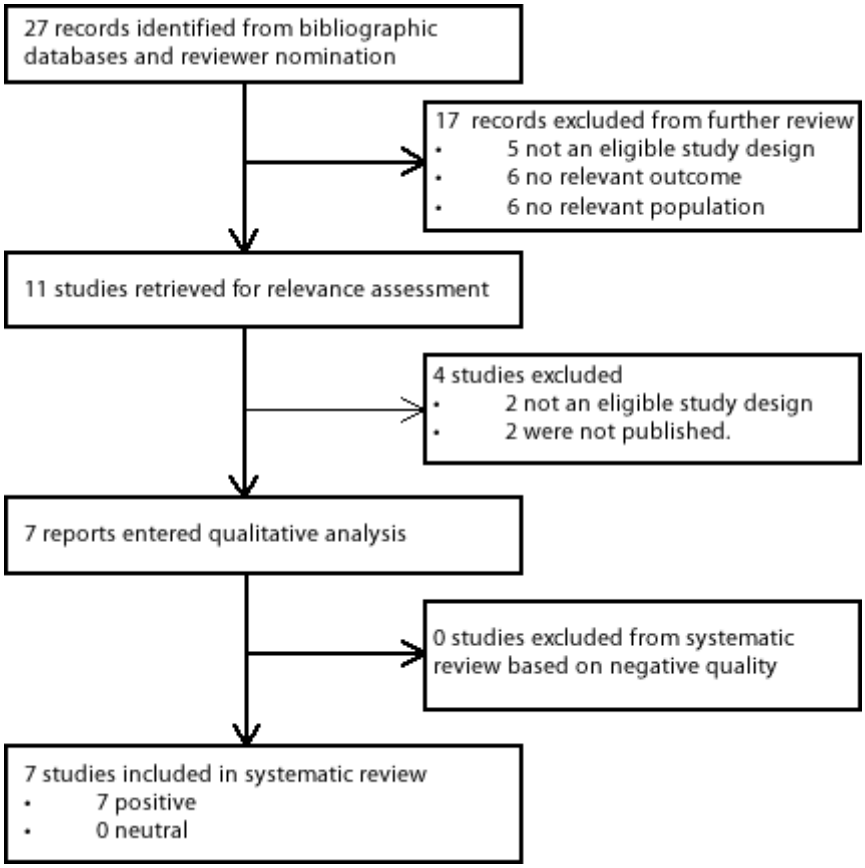
ginger preparation used within their study contained 8.5mg of active constituents per capsule; however, it was unclear whether this was independently analysed or from the manufacturers' analysis. None of the studies that used a powdered formulation reported an analysis of active constituents. The timing of doses did not vary greatly between studies, with the initial dose generally given +/-1 hour of the first chemotherapy session. Ryan et al.[30] was the exception to this in providing ginger supplementation for the three days prior to chemotherapy.

Five of the seven studies used standard anti-CINV medication in conjunction with ginger. In the two studies that did not use ginger as an adjuvant to standard therapy, ginger was compared to ondansetron and metoclopramide as a stand-alone treatment in a cross-over trial[28] or combined with standard anti-CINV treatment in the acute phase, but compared as a stand-alone treatment in the delayed phase of the study.[29] Participants in four of the seven of studies were adults of mixed gender, with the exceptions of Panahi et al.[31] and Manusirivithaya et al.[29] who studied females and Pillai et al.[32] who studied children.

2.5 Study results

The results of the included studies were mixed. Two of the seven studies reported no benefit,[18,33] three determined some benefit on measures of CINV (measures of either acute nausea[30,31] or both acute and delayed nausea and vomiting[32]) and two reported that ginger performed equally as well as metoclopramide (Table 2-2).[28,29] Zick et al.[18] found that higher doses (2g) of ginger had a negative effect on delayed-CINV in participants prescribed aprepitant (p=0.01).[16]

Figure 2-1 Flow of information for systematic review.



Sontakke et al.[28] found 2g of ginger effective in reducing acute CINV equal to metoclopramide; Pillai et al.[32] determined that 1-2g of ginger was effective in reducing the severity of both acute and delayed CINV by 37-47%; while Ryan et al.[30] reported that all doses used in the intervention successfully reduced symptoms of acute nausea by 0.16-0.44 on a 1-7 Likert scale in patients experiencing mild baseline-CINV (p=0.003), with 0.5g and 1g (p=0.017 and p=0.036, respectively) being the most effective doses; however, delayed nausea and quality of life were not affected by ginger supplementation. A 16% reduction in acute nausea during the first 6-24 hours post-chemotherapy was also found by Panahi et al.[31] using 1.5g of ginger (p=0.04).

Manusirivithaya et al.[29] reported that during the acute phase of chemotherapy, 1g of ginger did not further reduce CINV when combined with metoclopramide therapy. It did, however, perform equally to metoclopramide during the delayed phase (2-5 days post-chemotherapy). Zick et al.[18] and Fahimi et al.[33] found no additional benefit when ginger was used as an adjuvant therapy to standard nausea and emetic control.

A variety of tools were used to assess nausea and vomiting in the studies reviewed. Two studies measured symptoms using a modified version of the Morrow Assessment of Nausea and Emesis (MANE),[18,33] a validated instrument for assessing nausea in cancer patients[34]; Pillai et al.[32] employed the Edmonton Symptom Assessment Scale and the National Cancer Institute Guidelines for Nausea and Vomiting, respectively; two studies used an unspecified tool[28,29]; Panahi et al. [31] employed the Rhodes Index of Nausea, Vomiting, and Retching; and Ryan et al.[30] utilized a tool developed by Burish and Carey.[35]

Five of the seven studies specifically included patients receiving highly emetogenic chemotherapy regimens; however, while all being highly emetogenic regimens, there was little consistency in the agent and protocol used. The remaining two studies included patients undergoing combination chemotherapy containing agents with different degrees of emetogenicity.[18,30]

Table 2-1 Studies reviewed

Author	Study Design	Population	Type of cancer	Chemotherapy Protocol	Country	Level of evidence	Quality
Ryan et al. (2012)[30]	Randomized, double-blind, placebo-controlled, dose-finding trial	576 adult cancer patients. Mean age of 53 years. 93% women.	72% Breast, 28% Alimentary Genitourinary, Gynaecologic, Hematologic, Lung.	Not specified.	USA	II	Positive
Panahi et al. (2012)[31]	Randomized, open-label, pilot clinical trial	78 women. Mean age: 51.83 years.	Advanced breast cancer	Predominately, the TEC regimen (docetaxel, epirubicin, and Cyclophosphamide).	Iran	II	Positive
Pillai et al. (2011)[32]	Prospective, double-blind, randomized controlled trial	58 children, cancer patients. Mean age: 15 years. 40 men, 20 women.	Bone sarcoma.	Combination of cisplatin (40 mg/m^2/day) and doxorubicin (25 mg/m^2/day).	India	II	Positive
Fahimi et al. (2010)[33]	Randomized, cross-over, double-blinded, placebo-controlled trial	36 adult cancer patients. Mean age of 50.23 years. 10 women, 26 men.	50% Lung cancer, 50% Unspecified.	Cisplatin with at least one of the following agents: Etoposide, Docetaxel, Gemcitabine, Docetaxel, Vinorelbine Cyclophosphamide, Paclitaxel, Doxorubicin, 5-FU, Pemetrexed.	Iran	II	Positive
Zick et al. (2009)[18]	Randomized, double-blind, placebo-controlled trial	129 adult cancer patients. Mean age of 55.5-58 years. Approximately 75% female.	Unspecified.	Multiple regimens of varying emetogenicity.	USA	II	Positive
Manusirivithaya et al. (2004)[29]	Randomized, double-blind crossover trial	43 female cancer patients. Mean age of 43 years.	76% Ovary, 23% Cervix.	Cisplatin with one of the following agents: cyclophosphamide, ifosfamide, etoposide & bleomycin, 5-fluorouracil.	Thailand	II	Positive
Sontakke et al. (2003)[28]	Randomized, prospective, cross-over, double-blind trial	50 cancer patients. Median age of 46 years. 39 female, 11 male.	Unspecified.	Cyclophosphamide (500-1000mg) with at least one of the following agents: vincristine, methotrexate, 5-fluorouracil, actinomycin D.	India	II	Positive

Table 2-2 Study results

Author	Ginger regimen	Duration of intervention	Endpoint measured	Results and adherence	Comments
Ryan et al. (2012)[30]	Placebo, 0.5g ginger, 1g ginger or 1.5g ginger (6 capsules, combination of ginger and placebo).	Received regimen for 2 X 6 day periods. Measured for 3 X 4 day periods.	Primary objective: acute nausea. Secondary objectives: delayed nausea, anticipatory nausea, and quality of life.	All doses reduced acute nausea (p=0.003) but not delayed, using an assessment tool developed by Burish and Carey.[35] 77.4% of participants completed the trial (N=576/744), 83-93% adherence rate depending on treatment arm.	0.5 and 1g doses were most effective in reducing acute CINV. Largest study to date.
Panahi et al. (2012)[31]	1.5g (3 X 500mg)	4 days post-chemotherapy	Prevalence, score, and severity of nausea, vomiting, and retching	Reduction in nausea 6 to 24 hours post-chemotherapy (p = 0.04) using a simplified version of the Rhodes Index of Nausea, Vomiting, and Retching. All other measures were non-significant. 78% of participants completed the trial (N=78/100), 18 participants were withdrawn due to lack of adherence or were lost to follow-up.	Non-blinded. Sample group relatively homogenous compared to other studies in this review.
Pillai et al. (2011)[32]	1g ginger (6 X 167mg) or 2g (5 X 400mg) determined by participants weight, or placebo.	Received regimen for 3 days post-chemotherapy, measured symptoms for 10 days post-chemotherapy.	Incidence and severity of acute and delayed nausea and emetic events.	Reduction in moderate and severe acute nausea and emesis (p=0.003, p=0.002, respectively) and reduction in moderate and severe delayed nausea and emesis (p<0.001, p=0.022, respectively), using Edmonton's Symptom Assessment Scale and National Cancer Institute guidelines. 95% of participants completed the trial (N=57/60), 2 participants were withdrawn due to non-adherence with data collection protocol.	Experimental group contained a larger proportion of males, almost reaching statistical significance. Gender could influence susceptibility to nausea and vomiting.

Fahimi et al. (2010)[33]	1g (4 X 250mg) or placebo then crossed over.	2 X 3 day periods with a 3 week washout period in between.	Prevalence, severity and duration of acute and delayed nausea and emetic events.	No benefit in any measure of acute or delayed CINV, using MANE assessment tool. Prevalence: Day 1 (p=0.14). Day 2 (p=0.31). Day 3 (p=0.73). 72% of participants completed the trial (N=36/50), 13 participants were withdrawn due to non-adherence.	
Zick et al. (2009)[18]	1g (4 X 250mg, 4x placebo) or 2g (8 X 250mg) per day or placebo.	3 days post-chemotherapy	Primary objective: Severity and prevalence of delayed nausea and emetic events. Secondary objectives: Severity and prevalence of acute nausea and emetic events as well determine safety and blinding of study.	No benefit in any measure of acute or delayed CINV, using MANE assessment tool. Prevalence: Acute: p=0.86 Delayed: 0.16 Severity: Non-Appretiant group: Acute: p=0.47, Delayed: p=0.69. 80% of participants completed the trial (N=129/162). Authors reported 79% of participants reported consuming 80% of all study medication.	Delayed nausea was more severe in participants receiving 2g ginger with aprepitant. Blinding assessment found that participants were more likely to correctly determine which treatment group they were assigned to.
Manusirivithaya et al. (2004)[29]	1g ginger (4 X 250mg) or placebo then crossed over.	2 X 5 day periods with 3-4 week washout period in-between	Acute and delayed nausea and emetic events.	No benefit in acute nausea. Reduction in delayed CINV equal to standard treatment. 90% of participants completed the trial (N=43/48). No data on adherence rate specified.	The name of assessment tool in this study was not identified. In delayed phase, ginger was compared as a stand-alone treatment to metoclopramide, not placebo.
Sontakke et al. (2003)[28]	2g (4 X 500mg) ginger, crossed over with two control groups	3 X 24 hour periods with 21 days between sessions	Control of acute nausea and emesis.	Complete control of vomiting was achieved in 68% of patients with ginger, 64% with metoclopramide and 86% with ondansetron. Complete control of nausea was achieved in 62% of patients with ginger, 58% with metoclopramide and 86% with ondansetron. No data on withdrawals or adherence was specified.	Compared ginger to standard emetics as a standalone therapy. The name of assessment tool in this study was not identified.

Abbreviations: MANE, Morrow Assessment of Nausea and Emesis; CINV, Chemotherapy-induced Nausea and Vomiting.

2.5.1 *Adverse events and adherence*

Despite previous research indicating that ginger supplementation could theoretically cause excessive bleeding in susceptible patients due to the inhibition of platelet aggregation,[36] all adverse events that were attributed to the intervention were non-serious in nature. The most common reactions reported included heartburn, bruising or flushing, rash, and gastrointestinal discomfort. Adverse events were generally not significantly higher in the ginger group compared to the control group in any study.

Most studies (5/7) reported some degree of non-adherence during their investigations. Studies that included information regarding adherence found a rate between 75-90%.[18,30,31,33] The exact method for determining adherence was not stated in five of the seven studies, however, Ryan et al.[30]reported that adherence was measured by counting the amount of remaining pills at the end of each study cycle while Panahi et al.[31] measured self-reported adherence.

2.6 Discussion

The evidence is mixed in its support of ginger as an adjuvant or stand-alone treatment for CINV. Of the seven RCTs published to date; five reported favourable results while two were unfavourable. Of the five favourable studies, three studies reported ginger as improving some measure of CINV when combined with standard anti-CINV treatment, with Ryan et al.[30] and Panahi et al.[31] reporting a reduction in acute nausea and Pillai et al.[32] reporting a reduction in acute and delayed nausea and vomiting. The two other favourable studies found ginger reduced some measure of CINV equal to metoclopramide

but due to the lack of a placebo group in both studies, it is difficult to determine the clinical significance of these results[28,29]. This is due to the fact that in both of these trials, the percentage of individuals that reported symptoms in the ginger group was still within the predicted emetic risk for the chemotherapy regimen used and therefore, without a placebo group, it is difficult to determine the intervention's true impact. Results from positive trials have found ginger to reduce measures of CINV by 16-47% and while these findings need to be reconciled with the negative findings from other studies in this review, this magnitude of reduction could provide meaningful relief to patients experiencing CINV.

Using the NHMRC body of evidence assessment matrix, our review indicates that there is C level evidence for the use of ginger as an anti-nausea agent in this context. Therefore while there is some supporting evidence for its use, the considerable inconsistency in study methods and outcomes reported here reflect genuine uncertainty about its use in the chemotherapy setting. Until this uncertainty is resolved, professional opinion will continue to guide the healthcare team when choosing ginger as a treatment option.

2.6.1 *Confounding factors within current literature*

There are multiple factors that explain the mixed results reported in the literature. One possible explanation is that some ginger preparations have higher levels of certain active compounds when compared to the preparations used in other studies. Research investigating the concentration of active compounds in commercial ginger products

indicates that the levels of these compounds can vary greatly between products, demonstrating a need to analyse ginger interventions for their active compounds and to utilise standardised extracts rather than powdered formulations.[37,38] In order to improve the significance of future trials in this area, dose-finding studies using varied standardised extracts are required to determine the effective dose and preparation of ginger.

Recent studies have also determined that once a patient undergoing chemotherapy develops any form of nausea or vomiting (i.e. anticipatory, acute, delayed), regardless of the emetogenicity of that treatment, the likelihood of that patient experiencing nausea for the remainder of their treatment regimen is significantly higher and more difficult to treat with standard anti-CINV medication. [39] This is due to the complex aetiology of CINV, a response that is initiated by varying stimuli within the central and peripheral nervous systems. These include the effects of chemotherapy on both the central nervous system and gastrointestinal tract as well as the effect of sensory input (e.g. smell, sight) and the psychological conditions of the individual (e.g. fear, anxiety).[40] These stimuli activate peripheral and central nerve signals which are then received by the chemoreceptor trigger zone an area within the brain, which coordinates the body's emetic response base. Anticipatory nausea and vomiting is thought to be a conditioned response to previous chemotherapy exposure. Anticipatory CINV is mediated by the central nervous system and is caused by the coupling of neutral stimuli (such as the smell or sight of the hospital environment) with the undesirable effects of chemotherapy, which then results in the initially neutral stimuli eliciting a similar response to the cytotoxic treatment.[41] Since many studies in this review included patients who had previously experienced CINV, the

participants within these studies might have had an increased resistance to the intervention due to conditioning. This is of particular concern in the studies that used a cross-over design, as patients who were initially in the control group could have had established resistance to the intervention when subsequently crossed-over. Conducting statistical analysis to ensure that the sequence of intervention does not influence the results, as undertaken by Manusirivithaya et al.[29] and Zick et al.[18], will help monitor this effect. Alternatively, Roscoe and colleagues[30,42] were able to determine that a self-assessed susceptibility to nausea and vomiting by chemotherapy patients was a predictor of CINV and might be a viable method of screening in future trials.

Research has found that female patients are significantly more likely to experience CINV than their male counterparts.[43] The majority of studies (5/7) included a sample that was predominantly female, of which four studies reported benefits from ginger treatment. This suggests that gender could have influenced the patients' response to ginger treatment, possibly by decreasing the threshold at which CINV is experienced and thereby increasing the efficacy of anti-CINV treatments. In light of this, the null results reported by Fahimi et al.[33] could be partially explained by the male-dominant sample. In this study, the severity of nausea in both the intervention and control group was rated as low at all time points which indicates that the patients within this study could not have been experiencing CINV at a sufficiently high level of severity to have responded to anti-CINV intervention. This could also explain the results found by Pillai et al.[32] When the gender distributions between the control and treatment group were compared, there was a greater proportion of men within the experimental group compared to the control, which almost

reached statistical significance (p=0.055). This could have also resulted in the experimental group being more resistant to CINV compared to the control group regardless of ginger treatment. Therefore, similarly to anticipatory nausea, future trials should either include screening protocols or conduct statistical analyses to account for gender variations within the study sample.

Additionally, because of the subjective nature of nausea, direct comparison of findings can be difficult and therefore investigators should aim to use validated tools such as the MANE, which would ensure that results are both validated and easily comparable to other studies. It should be noted that the two studies that failed to find any benefit from ginger supplementation both used the MANE as the assessment tool, which suggests that the use of different assessment tools used within each study might have been a factor contributing to the mixed results of the reviewed literature.[18,33]

Another concern is that due to the distinctive aroma of ginger, it is important to ensure that studies are properly blinded. For example, Zick et al.[18] tested the effectiveness of the blinding in their investigation. While they had taken steps to ensure adequate blinding, the participants were able to discern the intervention group from the placebo at a statistically significant rate (p=0.01). To overcome this problem, Ryan et al.[30] utilised a combination of double encapsulation with a nitrogen cap to mask the odour and colour of the ginger. While this is an example of a potentially effective blinding technique, they did not test its effectiveness. Interestingly, Ryan et al's.[30] was one of the two studies that reported positive results when ginger was used as an adjuvant therapy; effective blinding

could, at least in part, help explain the disparity of results between studies within this review. Future clinical trials should incorporate more stringent blinding procedures to avoid a potential placebo or nocebo effect from occurring.

2.6.2 *Possible drug-interactions at high doses*

An interesting result reported within two studies in this review is that when subjects were given higher doses (1.5-2g) of ginger, there was a statistically significant decline in CINV control when compared to the participants that either received lower doses or the placebo. Zick et al.[18] reported that when subjects received a combination of 2g ginger plus aprepitant (an NK_1 inhibitor), the severity of delayed nausea increased when compared to control (p=0.01). Similarly, Ryan et al.[30] concluded that while all doses of ginger were effective in reducing acute CINV, 1.5g of ginger was less effective when compared to the 0.5g and 1g of ginger preparations. These findings corroborate previous studies in this field, which reported that higher doses of ginger were less effective when treating nausea from causes other than chemotherapy.[44,45] This led Zick et al.[18] to hypothesise that ginger reduces absorption of medication by increasing gastric emptying and intestinal motility, which has been demonstrated in animal models. However, research in human trials has not determined that ginger affects gastric emptying rates.[46,47] Another hypothesis is that ginger competitively interacts with the same receptors that standard anti-CINV medication acts upon; thereby reducing the binding rate of medications when used in combination.[30] Animal studies support this hypothesis, indicating that gingerols and shoagoals are able to bind to both 5-HT_3 and substance *P*

receptors, which are the receptors that medications such as aprepitant and ondansetron interact with.[48,49] It should also be noted that these studies showed that different ginger compounds bound to these receptors with varying strengths and therefore, different preparations of ginger could exert differing effects on nausea. This highlights further limitations in our current understanding in this area, as there are multiple active compounds in ginger that appear to be responsible for these interactions. This poses a significant limitation to the current research as the majority of studies, excluding Zick et al.[18] and Ryan et al.[30], used ginger preparations with unknown levels of these active constituents.

2.6.3 *Clinical Implications*

The feasibility of ginger supplementation has not been extensively or rigorously studied in chemotherapy populations. Fatigue, mouth sores and taste sensitivities are all common symptoms that chemotherapy patients experience while undergoing treatment. Given that some studies included in this review have used up to 8 capsules, consumed at multiple times throughout the day, this could place a significant burden on a population group who might already be compromised. Future research is required to investigate areas of practice such as participant tolerability and adherence to the intervention, in addition to its effect on quality of life and patient satisfaction with the intervention, in order to determine its real-world efficacy.

2.6.4 *Review limitations*

The exclusion of unpublished literature could have affected this review by introducing a publication bias; however, the two unpublished studies that were identified and excluded from this review both reported positive results and therefore this seems unlikely.[24,25]

2.7 Conclusion

Despite the widespread use of ginger in the treatment of nausea in other contexts such as gestational nausea, the current literature provides mixed support for the use of ginger as a standard part of anti-CINV control for patients undergoing chemotherapy. Hence standard recommendations for such use are premature. This review has discussed some of the limitations in our current understanding of the area and highlights the need for further investigation. In particular, issues regarding rigorous blinding procedures, patient screening, timing of the intervention to encompass the range of CINV, and ginger preparation should be considered in future research in this area. Our analysis of the evidence using NHMRC grading indicates that ginger could be useful for some patients but also that care needs to be taken in its application until further studies are conducted.

2.8 References

1. Bloechl-Daum B, Deuson RR, Mavros P, Hansen M, Herrstedt J. Delayed nausea and vomiting continue to reduce patients' quality of life after highly and moderately emetogenic chemotherapy despite antiemetic treatment. *J Clin Oncol.* Sep 20 2006;24(27):4472-4478.

2. Cohen L, de Moor CA, Eisenberg P, Ming EE, Hu H. Chemotherapy-induced nausea and vomiting: incidence and impact on patient quality of life at community oncology settings. *Support Care Cancer.* Could 2007;15(5):497-503.

3. Burke T, Wisniewski T, Ernst F. Resource utilization and costs associated with chemotherapy-induced nausea and vomiting (CINV) following highly or moderately emetogenic chemotherapy administered in the US outpatient hospital setting. *Supportive Care in Cancer.* 2011;19(1):131-140.

4. Isenring E, Cross G, Kellett E, Koczwara B, Daniels L. Nutritional Status and Information Needs of Medical Oncology Patients Receiving Treatment at an Australian Public Hospital. *Nutrition and Cancer.* 2010/01/25 2010;62(2):220-228.

5. Davidson W, Teleni L, Muller J, et al. Malnutrition and chemotherapy-induced nausea and vomiting : implications for practice. *Oncology Nursing Forum.* 2012.

6. Van Cutsem E, Arends J. The causes and consequences of cancer-associated malnutrition. *European journal of oncology nursing : the official journal of European Oncology Nursing Society.* 2005;9:S51-S63.

7. Tong HT, Isenring EA, Yates P. The prevalence of nutrition impact symptoms and their relationship to quality of life and clinical outcomes in medical oncology patients. *Supportive Care in Cancer.* 2008;17(1):83-90.

8. Eisenberg DM, Davis RB, Ettner SL, et al. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA : the journal of the American Medical Association.* Nov 11 1998;280(18):1569-1575.

9. Yamahara J, Huang QR, Li YH, Xu L, Fujimura H. Gastrointestinal motility enhancing effect of ginger and its active constituents. *Chemical & pharmaceutical bulletin.* Feb 1990;38(2):430-431.

10. Wu KL, Rayner CK, Chuah SK, et al. Effects of ginger on gastric emptying and motility in healthy humans. *Eur J Gastroenterol Hepatol.* Could 2008;20(5):436-440.

11. Riyazi A, Hensel A, Bauer K, Geissler N, Schaaf S, Verspohl EJ. The effect of the volatile oil from ginger rhizomes (Zingiber officinale), its fractions and isolated compounds on the 5-HT3 receptor complex and the serotoninergic system of the rat ileum. *Planta Med.* Apr 2007;73(4):355-362.

12. Linnane AW, Kios M, Vitetta L. Coenzyme Q(10)--its role as a prooxidant in the formation of superoxide anion/hydrogen peroxide and the regulation of the metabolome. *Mitochondrion.* Jun 2007;7 Suppl:S51-61.

13. Linnane A, Kios M, Vitetta L. Healthy aging: regulation of the metabolome by cellular redox modulation and prooxidant signaling systems: the essential

roles of superoxide anion and hydrogen peroxide. *Biogerontology.* 2007;8(5):445-467.

14. Sharma SS, Kochupillai V, Gupta SK, Seth SD, Gupta YK. Antiemetic efficacy of ginger (Zingiber officinale) against cisplatin-induced emesis in dogs. *J Ethnopharmacol.* Jul 1997;57(2):93-96.

15. Sharma SS, Gupta YK. Reversal of cisplatin-induced delay in gastric emptying in rats by ginger (Zingiber officinale). *J Ethnopharmacol.* Aug 1998;62(1):49-55.

16. Ernst E, Pittler MH. Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials. *British Journal of Anaesthesia.* March 1, 2000 2000;84(3):367-371.

17. Natural Medicines Comprehensive Database. Therapeutic Research Faculty; 1995-2012. www.naturaldatabase.com. Accessed 28/03/2012.

18. Zick S, Ruffin M, Lee J, et al. Phase II trial of encapsulated ginger as a treatment for chemotherapy-induced nausea and vomiting. *Supportive Care in Cancer.* 2009;17(5):563-572.

19. MIMS Australia. Ondansetron. In: MIMS. 2012; Available at: http://www.mims.com.au. Accessed February 22, 2013.

20. MIMS Australia. Dexamethasone. In: MIMS. 2012; Available at: http://www.mims.com.au. Accessed February 22, 2013.

21. MIMS Australia. Ginger. In: MIMS. 2012; Available at: http://www.mims.com.au.

Accessed February 22, 2013.

22. National Health and Medical Research Council. NHMRC additional levels of evidence and grades for recommendations for developers of guidelines. *Commonwealth of Australia: National Health and Medical Research Council* 2009.

23. ADA. Evidence Analysis Manual: Steps in the ADA Evidence Analysis Process. American Dietetic Association2010.

24. Pecoraro A, Patel J, Guthrie T, Ndubisi B. Efficacy of ginger as an adjunctive anti-emetic in acute chemotherapy-induced nausea and vomiting. *ASHP Midyear Clinical Meeting.* 1998;33:P-429E.

25. Pace J, Conlin D. Oral ingestion of encapsulated ginger and reported self-care actions for the relief of chemotherapy-associated nausea and vomiting. *Dissertation Abstracts International.* 1987;47(8):3297-B.

26. Levine ME GM, Koch SY, Voss AC, Stern RM, Koch KL. Protein and ginger for the treatment of chemotherapy-induced delayed nausea. . *The Journal of Alternative and Complementary Medicine.* 2008;14(5):545-551.

27. Meyer K, Schwartz J, Crater D, Keyes B. Zingiber officinale (ginger) used to prevent 8-Mop associated nausea. *Dermatology nursing / Dermatology Nurses' Association.* 1995;7(4):242-244.

28. SONTAKKE, S., THAWANI, V., NAIK, S. M. Ginger as an antiemetic in nausea and vomiting induced by chemotherapy: A randomized, cross-over,

double blind study. Vol 35. Pondicherry, INDE: Indian Pharmacological Society; 2003.

29. Manusirivithaya S, Sripramote M, Tangjitgamol S, et al. Antiemetic effect of ginger in gynecologic oncology patients receiving cisplatin. *Int J Gynecol Cancer*. Nov-Dec 2004;14(6):1063-1069.

30. Ryan JL, Heckler CE, Roscoe JA, et al. Ginger (Zingiber officinale) reduces acute chemotherapy-induced nausea: a URCC CCOP study of 576 patients. *Support Care Cancer*. Jul 2012;20(7):1479-1489.

31. Panahi Y, Saadat A, Sahebkar A, Hashemian F, Taghikhani M, Abolhasani E. Effect of Ginger on Acute and Delayed Chemotherapy-Induced Nausea and Vomiting: A Pilot, Randomized, Open-Label Clinical Trial. *Integr Cancer Ther*. Feb 7 2012.

32. Pillai AK, Sharma KK, Gupta YK, Bakhshi S. Anti-emetic effect of ginger powder versus placebo as an add-on therapy in children and young adults receiving high emetogenic chemotherapy. *Pediatric blood & cancer*. Feb 2011;56(2):234-238.

33. Fahimi F, Khodadad K, Amini S, et al. Evaluating the Effect of Zingiber Officinalis on Nausea and Vomiting in Patients Receiving Cisplatin Based Regimens. *Iranian Journal of Pharmaceutical Research.* 2010;10(2):379-384.

34. Rhodes VA, McDaniel RW. Nausea, Vomiting, and Retching: Complex Problems in Palliative Care. *CA: A Cancer Journal for Clinicians.* 2001;51(4):232-248.

35. Burish TG, Carey MP, Krozely MG, Greco FA. Conditioned side effects induced by cancer chemotherapy: Prevention through behavioral treatment. *Journal of Consulting and Clinical Psychology.* 1987;55(1):42-48.

36. K.C S. Isolation and effects of some ginger components on platelet aggregation and eicosanoid biosynthesis. *Prostaglandins, Leukotrienes and Medicine.* 1986;25(2–3):187-198.

37. Schwertner HA, Rios DC, Pascoe JE. Variation in concentration and labeling of ginger root dietary supplements. *Obstet Gynecol.* Jun 2006;107(6):1337-1343.

38. Schwertner HA, Rios DC. High-performance liquid chromatographic analysis of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in ginger-containing dietary supplements, spices, teas, and beverages. *J Chromatogr B Analyt Technol Biomed Life Sci.* Sep 1 2007;856(1-2):41-47.

39. Roscoe JA, Morrow GR, Aapro MS, Molassiotis A, Olver I. Anticipatory nausea and vomiting. *Support Care Cancer.* Oct 2011;19(10):1533-1538.

40. Wender RH. Do current antiemetic practices result in positive patient outcomes? Results of a new study. *American Journal of Health-System Pharmacy.* January 1, 2009 2009;66(1 Supplement 1):S3-S10.

41. Stockhorst U, Steingrueber H-J, Enck P, Klosterhalfen S. Pavlovian conditioning of nausea and vomiting. *Autonomic Neuroscience.* 2006;129(1–2):50-57.

42. Roscoe JA, Morrow GR, Colagiuri B, et al. Insight in the prediction of chemotherapy-induced nausea. *Supportive Care in Cancer.* 2010;18(7):869-876.

43. Osoba D, Zee B, Pater J, Warr D, Latreille J, Kaizer L. Determinants of postchemotherapy nausea and vomiting in patients with cancer. Quality of Life and Symptom Control Committees of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol.* Jan 1997;15(1):116-123.

44. Arfeen Z, Owen H, Plummer JL, Ilsley AH, Sorby-Adams RA, Doecke CJ. A double-blind randomized controlled trial of ginger for the prevention of postoperative nausea and vomiting. *Anaesthesia and intensive care.* 1995;23(4):449-452.

45. Lien H-C, Sun WM, Chen Y-H, Kim H, Hasler W, Owyang C. Effects of ginger on motion sickness and gastric slow-wave dysrhythmias induced by circular vection. *Am J Physiol Gastrointest Liver Physiol.* 2003;284(12576305):481-489.

46. Stewart JJ, Wood MJ, Wood CD, Mims ME. Effects of ginger on motion sickness susceptibility and gastric function. *Pharmacology.* 1991;42(2):111-120.

47. Phillips S, Hutchinson S, Ruggier R. Zingiber officinale does not affect gastric emptying rate. A randomised, placebo-controlled, crossover trial. *Anaesthesia.* Could 1993;48(5):393-395.

48. Abdel-Aziz H, Windeck T, Ploch M, Verspohl EJ. Mode of action of gingerols and shogaols on 5-HT3 receptors: binding studies, cation uptake by the receptor channel and contraction of isolated guinea-pig ileum. *Eur J Pharmacol.* Jan 13 2006;530(1-2):136-143.

49. Qian QH, Yue W, Wang YX, Yang ZH, Liu ZT, Chen WH. Gingerol inhibits cisplatin-induced vomiting by down regulating 5-hydroxytryptamine, dopamine and substance P expression in minks. *Arch Pharm Res.* Apr 2009;32(4):565-573.

Chapter 3. Is ginger beneficial for nausea and vomiting? An update of the literature.

The manuscript included in Chapter 3 was the result of an invitation by the editors of *Current Opinion in Supportive & Palliative Care* (2014 Impact Factor: 1.656; 47 of 89 Health Care Science & Service) to provide an update on the clinical data regarding the use of ginger for nausea from any stimuli (e.g. CINV as well as morning sickness and motion sickness). This chapter provides an update to the systematic literature review included in Chapter 2 by discussing clinical trials that were conducted since the literature search date and provides a broader overview of the recent evidence for the use of ginger for nausea.

Citation:

Marx W, Kiss N, Isenring L. Is ginger beneficial for nausea and vomiting? An update of the literature. Current Opinion in Supportive and Palliative Care. 2015;9(2):189-95. doi: 10.1097/spc.0000000000000135. PubMed PMID: 01263393-201506000-00018.

3.1 Abstract

Purpose of review: Nausea and vomiting can pose a significant burden to patients in a variety of clinical settings. Previous evidence suggests ginger could be an effective treatment for these symptoms; however, current evidence has been mixed. This review discusses recent clinical trials that have investigated ginger as a treatment for multiple types of nausea and vomiting. In addition, the potential mechanisms of action of ginger will be discussed.

Recent findings: This review identified nine studies and seven reviews that investigated ginger for morning sickness, post-operative nausea and vomiting, chemotherapy and anti-retroviral induced nausea and vomiting. All studies reported ginger to provide a significant reduction in nausea and vomiting; however, the clinical relevance of some studies is less certain. Common limitations within the literature include the lack of standardised extracts, poorly controlled or blinded studies, and limited sample size. In addition, recent evidence has provided further support for 5-HT_3 receptor antagonism as a mechanism by which ginger could exert its potentially beneficial effect on nausea and vomiting.

Summary: The results of studies in this review suggest that ginger is a promising treatment for nausea and vomiting in a variety of clinical settings and possesses a clinically relevant mechanism. However, further studies are required to address the limitations in the current clinical literature before firm recommendations for its use can be made.

3.2 Key Points

- The active constituents within ginger have been reported to exert 5-HT_3 receptor antagonism, a clinically relevant mechanism for treating nausea and vomiting.
- Ginger has demonstrated preliminary efficacy in reducing nausea and/or vomiting in response to a variety of stimuli including surgery, pregnancy, chemotherapy, and more recently, antiretroviral therapy.
- While the included studies generally reported statistically significant reductions in nausea and vomiting measures, the clinical significance of these results were mixed.
- Current limitations in clinical studies include lack of standardised extracts, poorly controlled or blinded studies, and limited sample size.
- To date, studies have reported few adverse events associated with ginger supplementation; however, further studies are required to assess its safety profile.

3.3 Introduction

Nausea and vomiting can pose a significant burden to the patient, resulting in reduced quality of life, further medical complications, malnutrition, and in some settings, could potentially result in treatment disruption and stoppage.[1] Due to the high prevalence of nausea and/or vomiting in settings such as during chemotherapy and pregnancy, there has been considerable research interest in a variety of adjuvant therapies aimed at improving symptom control.

Ginger has had a long history of use in traditional systems of medicine for gastrointestinal complaints and continues to be commonly used as an anti-nausea

agent.[2] Ginger contains a wide-array of bioactive compounds which have been investigated for their effects on nausea and vomiting. Multiple potential mechanisms of action have been identified including 5-HT_3 receptor antagonism, anti-inflammatory properties and the modulation of gastrointestinal motility.[3] Clinical studies have also reported promising results and ginger has now been investigated as a treatment for a several types of nausea including motion sickness, post-operative nausea and vomiting, morning sickness, and chemotherapy-induced nausea and vomiting.[4]

Due to the continued investigation in this area, the aim of this review is to discuss recently published clinical studies that have investigated the use of ginger as a treatment for nausea and vomiting in any setting. Secondly, this review will provide an update regarding results of recent research on the mechanisms by which ginger could exert its potentially beneficial effect.

3.4 Methodology

A systematic search of the literature was conducted using the following databases: Medline, CINAHL, and the Cochrane Library. Search queries were limited to manuscripts published from 2013 until December 2014. Due to the limited time frame, the search query, (“ginger” AND “nausea”), was left broad so as to capture all relevant articles. Reference lists of retrieved manuscripts were also examined for additional publications.

Inclusion criteria for this review were as follows: 1) manuscripts published in English 2) study examined ginger as the primary intervention, and 3) investigated the effect of the intervention on nausea and/or vomiting outcomes or on mechanisms involved

in the generation of nausea and/or vomiting. Clinical studies that investigated ginger for nausea and/or vomiting in response to any stimuli were included.

3.5 Clinical efficacy

The search retrieved 16 articles related to the clinical efficacy of ginger in relation to nausea and vomiting. These include seven reviews,[1, 4-9] three of which also conducted a meta-analyses,[6-8] and nine original studies that investigated either the safety or efficacy of ginger as a treatment of nausea and/or vomiting.[10-18] For the clarity of this manuscript, the following discussion has been categorised by the type of nausea and/vomiting that was investigated. Clinical trials are also included in an extraction table (Table 3-1).

3.5.1 *Morning sickness*

Four systematic reviews were retrieved, two of which also performed a meta-analysis of included studies.[5-7, 9] Although the inclusion criteria varied between reviews, all four reviews reported ginger to be effective in reducing symptoms of nausea and/or vomiting.

Viljoen et al.[6] conducted a systematic review which included 12 studies, comprising 1278 participants. A particular strength of this study is that instead of restricting the inclusion criteria to increase the homogeneity, it categorised studies according to the comparison intervention (i.e placebo, metoclopramide, vitamin B6 and dimenhydrinate) which was then discussed separately. The majority of studies review reported either a beneficial effect when compared to placebo or either an equivalent or

superior effect when compared to metoclopramide, vitamin B6 and dimenhydrinate.[6] When possible, a meta-analysis was conducted but due to the significant heterogeneity in study designs and reporting of outcomes, generally only two studies per analysis was included and so the significance of these results is limited.

This study also analysed the adverse events and risk of spontaneous abortion and while the number of studies analysed for each adverse effect was small, the analysis found no significant difference in any adverse effect or risk of spontaneous abortion between ginger and placebo.[6] Heitmann et al.[13] also explored the relationship between ginger usage during pregnancy and risk of congenital malformations in a cohort study. From the 1020 women that reported consuming ginger during pregnancy, no statistically significant increase in multiple adverse outcomes (including stillbirth or perinatal death, preterm birth, low birth weight, low apgar score) was detected. Although an increased risk of non-severe vaginal bleeding was reported (7.8 % vs. 5.8 %, p=0.007).

The two clinical trials that were identified in our literature search both reported ginger to be effective in improving measures of nausea and vomiting. The first study was conducted by Javadi et al.[18] who conducted an open-label study that compared the efficacy of 1g ginger and vitamin B6 in 95 women. Saberi et al.[15] conducted a randomised controlled trial which compared the efficacy of ginger or acupressure to a control group. Both studies found ginger to be effective in significantly reducing the severity of nausea and vomiting from moderate severity to mild severity. However, neither study was blinded which presents a significant study limitation.

3.5.2 *Post-operative nausea and vomiting*

Four clinical studies were included in this review that investigated the effect of ginger for post-operative nausea and vomiting. All studies had relatively large sample sizes (*N*=100-303), making these the largest studies to date that have investigated post-operative nausea and vomiting.

Mandal et al.[17] investigated the effect of ginger on 100 participants undergoing a range of surgeries in the ambulatory setting and reported significantly reduced severity of nausea and vomiting symptoms four and six hours post-surgery as well as significant reductions in the incidence of post-operative nausea and vomiting at multiple time points within the 18 hours post-surgery.. Montezzari et al.[11] also investigated ginger as a treatment for post-operative nausea and vomiting at two, four and six hours post-surgery in patients undergoing diverse surgeries and reported a modest benefit in nausea reduction at two hours. As previous studies have been primarily in patients receiving gynaecological surgeries, these results add to the literature by demonstrating potential efficacy in a broader range of surgeries. However, the mean severity of post-operative nausea and vomiting reported in these studies was low for both the intervention and placebo groups and so the reported difference in severity could not be clinically significant.

In addition to post-operative nausea and vomiting, Kalava et al.[14] investigated the use of 1g ginger supplementation for intraoperative nausea and vomiting in 239 participants receiving elective caesarean section. The results showed a statistically

significant reduction in the frequency of intraoperative nausea but not intraoperative vomiting or post-operative nausea or vomiting. However, the mean difference in nausea between groups (0.396 on a 10 point visual analogue scale) is unlikely to translate into a meaningful difference to the patient. Despite this, this is the first study to investigate the effect of ginger on intra-operative nausea and vomiting and as this is a significant issue during particular surgeries, the results of this study suggest that this is an area worth further investigation.

Table 3-1. Extraction table of included clinical trials investigating ginger for nausea and vomiting.

Name	Type of nausea and/or vomiting	Study design	Intervention	Dosage	Outcomes	Assessment form	Results	Comments
Javadi et al. (2013)	Morning Sickness	N=95 women Duration: 4 days Design: open-label clinical trial	Ginger or vitamin B6	1g (4x250mg)	Occurrence and frequency of nausea Occurrence of retches and vomiting	MPUQE scoring system	Both ginger and vitamin B6 significantly reduced all outcomes compared to pre-treatment Treatment effect of interventions was equal	No placebo group Not blinded
Saberi et al. (2013)	Morning Sickness	N=159 Duration: 7 days Design: Randomised controlled trial	Ginger or P6 acupressure	750mg (3x250mg)	Nausea score Vomiting score Retching score Total score	Rhodes Index of Nausea, Vomiting and Retching	Nausea score was reduced by 48% Vomiting score was reduced by 52% Total score was reduced by 49% in ginger group Ginger group has greater reductions in all scores compared to control and acupressure group	Acupressure scores not included in this table Not blinded
Kalava et al. (2013)	Post- and intra-operative nausea and vomiting	N=239 women Duration: During and 24 hours post-surgery Design: Double-blind randomized placebo controlled trial	Ginger	2g (2x1g)	Intraoperative incidence and frequency of nausea and vomiting Postoperative incidence and severity of nausea and vomiting	Three Item visual analogue scale	Intra-operative symptoms: Reduced nausea frequency (p= 0.023) but not incidence. No significant effect on vomiting. Post-operative symptoms: No statistically significant difference in any outcome	

Mandal et al. (2014)	Post-operative nausea and vomiting	N=100 Duration: 18 hours post operation Design: Double-blind randomized placebo controlled trial	Ginger	1g (2x500mg)	Frequency and severity of nausea, vomiting and retching	Frequency of symptoms and medications used was recorded using tool developed by Bellville et al.[15] Severity of symptoms measured using a one item visual analogue scale	Reduce frequency of nausea, vomiting and retching at 2, 4, 6, 8 and 12 hours post-surgery ($p<0.05$) Reduced severity of nausea and vomiting at four and six hours post operation ($p<0.05$) Use of rescue medications was significantly lower in the ginger group ($P<0.05$)	
Montazeri et al. (2013)	Post-operative nausea and vomiting	N=160 Duration: Design: Double-blind randomized placebo controlled trial	Ginger	1g (4x250mg)	Frequency of retching and vomiting Severity of nausea	Visual analogue scale	Reduced severity and frequency of nausea at 2 hours post-surgery but not at 4 and 6 hours ($p=0.04$, 0.05, respectively). No significant difference in frequency of vomiting or retching	
Hunt et al (2013)	Post-operative nausea and vomiting	N=301 Duration: Design: Randomised, placebo-controlled trial	Ginger essential oil Essential oil blend (ginger, cardamom, spearmint, peppermint) Isopropyl alcohol	N/A	Severity of nausea Anti-emetic medication request	Visual analogue scale	Reduced number of requests for anti-emetic medication ($p=0.001$) Reduced severity of nausea ($p=0.002$)	Essential oil blend also significantly improved outcomes
Montazeri et al. (2013)	Chemotherapy-induced nausea and vomiting	N=44 Duration: Two chemotherapy cycles Design: randomized cross-over trial	Ginger	1g (4x250mg)	Frequency and severity of acute nausea and vomiting Retching frequency Rescue medication usage	Two item visual analogue scale	Reduced severity and frequency of nausea and vomiting ($p=0.001$)	Delayed nausea not assessed

Dabaghzadeh et al. (2014)	Antiretroviral-induced nausea and vomiting	N=102 Duration: 14 days Design: Double-blind randomized placebo controlled trial	Ginger	1g (4x250mg)	Incidence of any severity of nausea and vomiting Incidence of mild, moderate, and severe nausea and vomiting	Visual analogue scale	Reduced incidence of total nausea and vomiting in ginger group ($p<0.001$) Reduced incidence of mild, moderate, and severe nausea and vomiting in ginger group ($p = 0.02, 0.04$ and 0.001, respectively)	

Hunt et al.[10] reported ginger as an aromatherapy, either stand-alone or when combined with other essential oils, significantly reduced postoperative-nausea when compared to a saline control. Data regarding the baseline severity of nausea in patients was not reported and so the effect of ginger on different severities of nausea is unclear. In addition, as the method of delivery was via inhalation, it is likely that the mechanism of action could differ from ingested ginger and so the results of this study could not be able to be directly compared to studies that used ginger in supplement form.

3.5.3 *Chemotherapy-induced nausea and vomiting*

Two systematic literature reviews were identified which examined the use of ginger for chemotherapy-induced nausea and vomiting (CINV), predominately in patients receiving moderately and highly emetogenic chemotherapy regimens.[1, 8] Both reviews concluded that the current evidence for the use of ginger during chemotherapy is mixed and that further trials are needed to address existing limitations.

Limitations that were specific to CINV include the use of anti-emetic regimens that are not in line with current anti-emetic guidelines, and the lack of control or consideration for prognostic factors that could have influenced risk of nausea and vomiting (e.g. history of alcohol intake and motion sickness).

Lee et al.[8] performed a meta-analysis of included studies and while no effect on incidence of acute nausea and vomiting, and severity of acute nausea was reported, only two to three studies were included per analyses which limits the strength of these conclusions.

One clinical study was also identified which reported significant reductions in the frequency and incidence of acute nausea and vomiting in patients receiving 1g of ginger when compared to placebo.[12] While this trial adds to the promising literature regarding the clinical application of ginger in the chemotherapy setting, the limitations identified in the aforementioned reviews are still present in this study.

3.5.4 *Antiretroviral-induced nausea and vomiting*

Dabaghzadeh et al.[16] conducted a randomised controlled trial on the effect of ginger on nausea and vomiting induced by antiretroviral medication in 102 HIV positive participants. The investigators reported that 1g of ginger over 2 weeks significantly reduced the frequency and severity of nausea and the frequency of vomiting (p=0.001). Furthermore, the magnitude of effect was considerable with large difference in reported outcomes between the intervention and placebo group. To our knowledge, this is the first report of ginger being used as an anti-nausea and vomiting agent during antiretroviral therapy and therefore, demonstrates another setting where ginger could be of benefit and due to the promising results, warrants further study.

3.6 Mechanisms of action

Our search retrieved two original studies and two review articles that had investigated the mechanisms of action of ginger and its bioactive compounds in relation to pathways involved in nausea and vomiting.[19-22]

There are several mechanisms by which ginger could reduce nausea and vomiting symptoms; however, 5-HT_3 receptor antagonism is arguably one of the strongest

candidates for its primary mechanism. Previous work has demonstrated that bioactive compounds exhibit 5-HT_3 antagonism in murine cell lines but while these studies have provided strong support for ginger interacting with these receptors, Walstab et al.[21] has advanced this area by investigating this effect in human 5-HT_3 receptors and confirmed the following findings. First, this study has demonstrated that both ginger extracts and the compounds, 6-gingerol and 6-shogaol, non-competitively inhibited 5-HT_3 receptor activation. This provides both support for ginger interacting with the 5-HT_3 receptors in humans but also provides additional evidence that these compounds bind to a currently unknown binding site, distinct from other types of 5-HT_3 receptor antagonists. As Walstab et al.[21] noted, this could allow for potentially synergistic inhibition of 5-HT_3 signalling when combined with standard 5-HT_3 antagonists (e.g ondansetron, a common anti-emetic during chemotherapy and surgery). In addition, it was also noted that a CO_2 extract had a greater inhibition potency than what would be expected from 6-gingerol and 6-shogaol alone which suggests other compounds could also play a role.

Jin et al.[22] also investigated the effect of 6-gingerol and 6-shogaol as well as an additional compound, zingerone, on 5-HT_3 signalling and while the methodology differed to the studies conducted by Walstab et al.[21], the study also demonstrated an inhibition of 5-HT_3 signalling by these compounds. The finding that zingerone also exerted an effect on 5-HT_3 signalling confirms the results of Walstab et al.[21] by demonstrating an additional bioactive compound.

3.7 Discussion and future directions

Despite the limitations that have been discussed in the included reviews, the current literature regarding the use of ginger as a treatment for nausea and vomiting is promising as all studies in this review reported some degree of improvement in symptoms.

However, before recommendations can be made regarding its use in clinical practice, the existing limitations need to be addressed. These include the lack of use of validated assessment tools for nausea and vomiting, the significant heterogeneity of study designs and the use of unstandardized ginger supplements, which could account for the sometimes conflicting results and makes comparison between studies difficult. The continued use of unstandardized supplements, in particular, poses a significant issue when comparing studies with conflicting results due to the significant variation in bioactive compounds that can occur between different ginger products. The implementation of standardised extracts and/or quantification of bioactive compounds within ginger products are steps that would address this issue and that should be considered in future studies in this area.

In addition to the need for continued research into the efficacy of ginger for nausea, larger studies are required to assess the potential contraindications of ginger supplementation. These include general concerns such as the potential effect of ginger on platelet aggregation which could affect multiple patient populations as well as more population-specific concerns such as the potential risk of aspiration from oral ginger supplementation prior to surgery.

One important factor to be considered when appraising an intervention is the *clinical* significance of the results. If results are found to be statistically significant, two questions need to be considered: 1) can these results be generalised to a real world setting? And 2) are these results likely to significantly influence clinical outcomes?

This review has commented on both the statistical and clinical significance of the included studies and has found that the results of these studies provide mixed responses to these questions. The identified clinical studies that have investigated post-operative nausea and vomiting and CINV, for example, have generally reported statistically significant reductions in measures of nausea and vomiting; however, the magnitude of this reduction was generally small and so less likely to result in a substantial benefit to the patient.[11, 14, 17] The study conducted by Dabaghzadeh et al,[16] in contrast, demonstrated a considerable difference between the intervention and placebo group and if further studies report similar findings, this is likely to provide a clinically significant benefit to the patient.

In order to improve the interpretation of clinical significance, the inclusion of quality of life measures, for example the Functional Living Index Emesis – 5 day recall tool, in future studies would provide insight into the effect that the patients symptoms have on their day-to-day experience.

3.8 Conclusion

In summary, despite existing limitations, the clinical evidence included in this review suggests ginger could be an effective treatment for nausea and vomiting in multiple settings. However, further studies are required to address these limitations and to investigate the safety profile in each population. In addition, recent research has provided further evidence that the compounds within ginger exert 5-HT_3 receptor antagonism which suggests a clinically relevant mechanism for the treatment of nausea and vomiting, particularly for symptoms experienced during chemotherapy and surgery.

3.9 Annotated References

1. Marx WM, Teleni L, McCarthy AL, Vitetta L, McKavanagh D, Thomson D, et al. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. Nutrition Reviews. 2013;71(4):245-54.

   **Our previous review provides an in-depth discussion regarding the current limitations in clinical research on ginger and chemotherapy-induced nausea and vomiting. Many of the limitations discussed are applicable to research that has investigated other types of nausea and vomiting.*

2. Kennedy D, Lupattelli A, Koren G, Nordeng H. Herbal medicine use in pregnancy: results of a multinational study. BMC Complementary and Alternative Medicine. 2013;13(1):355.

3. Marx WM, Ried K, McCarthy AL, Vitetta L, Sali A, McKavanagh D, et al. Ginger - mechanism of action in chemotherapy-induced nausea and vomiting: a review. (In Press). Crit Rev Food Sci Nutr. 2015.

4. Palatty PL, Haniadka R, Valder B, Arora R, Baliga MS. Ginger in the prevention of nausea and vomiting: a review. Crit Rev Food Sci Nutr. 2013;53(7):659-69.

5. Ding M, Leach M, Bradley H. The effectiveness and safety of ginger for pregnancy-induced nausea and vomiting: A systematic review. Women Birth. 2012 Aug 27.

6. Viljoen E, Visser J, Koen N, Musekiwa A. A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting. Nutr J. 2014;13:20.

***Viljoen provides a detailed overview of ginger for pregnancy-induced nausea and vomiting including analyses and discussion regarding safety.*

7. Thomson M, Corbin R, Leung L. Effects of ginger for nausea and vomiting in early pregnancy: a meta-analysis. Journal of the American Board of Family Medicine : JABFM. 2014 Jan-Feb;27(1):115-22.

8. Lee J, Oh H. Ginger as an antiemetic modality for chemotherapy-induced nausea and vomiting: a systematic review and meta-analysis. Oncol Nurs Forum. 2013 Mar;40(2):163-70.

9. Dante G, Pedrielli G, Annessi E, Facchinetti F. Herb remedies during pregnancy: a systematic review of controlled clinical trials. J Matern Fetal Neonatal Med. 2013 Feb;26(3):306-12.

10. Hunt R, Dienemann J, Norton HJ, Hartley W, Hudgens A, Stern T, et al. Aromatherapy as treatment for postoperative nausea: a randomized trial. Anesthesia and analgesia. 2013 Sep;117(3):597-604.

11. Montazeri AS, Hamidzadeh A, Raei M, Mohammadiun M, Montazeri AS, Mirshahi R, et al. Evaluation of Oral Ginger Efficacy against Postoperative Nausea and Vomiting: A Randomized, Double - Blinded Clinical Trial. Iran Red Crescent Med J. 2013 Dec;15(12):e12268.

12. Montazeri AS, Raei M, Ghanbari A, Dadgari A, Hamidzadeh A. Effect of herbal therapy to intensity chemotherapy-induced nausea and vomiting in cancer patients. Iran Red Crescent Med J. 2013 Feb;15(2):101-6.

13. Heitmann K, Nordeng H, Holst L. Safety of ginger use in pregnancy: results from a large population-based cohort study. Eur J Clin Pharmacol. 2013;69(2):269 - 77.

14. Kalava A, Darji SJ, Kalstein A, Yarmush JM, SchianodiCola J, Weinberg J. Efficacy of ginger on intraoperative and postoperative nausea and vomiting in elective cesarean section patients. Eur J Obstet Gynecol Reprod Biol. 2013 Jul;169(2):184-8.

15. Saberi F, Sadat Z, Abedzadeh-Kalahroudi M, Taebi M. Acupressure and Ginger to Relieve Nausea and Vomiting in Pregnancy: a Randomized Study. Iranian Red Crescent Medical Journal. 2013 08/10/accepted;15(9):854-61.

16. Dabaghzadeh F, Khalili H, Dashti-Khavidaki S, Abbasian L, Moeinifard A. Ginger for prevention of antiretroviral-induced nausea and vomiting: a randomized clinical trial. Expert Opin Drug Saf. 2014 Jul;13(7):859-66.

**Dabaghzadeh is the first study that has investigate the effect of ginger on anti-retroviral induced nausea and vomiting*

17. Mandal P, Das A, Majumdar S, Bhattacharyya T, Mitra T, Kundu R. The efficacy of ginger added to ondansetron for preventing postoperative nausea and vomiting in ambulatory surgery. Pharmacognosy research. 2014 Jan;6(1):52-7.

18. Haji Seid Javadi E, Salehi F, Mashrabi O. Comparing the Effectiveness of Vitamin B6 and Ginger in Treatment of Pregnancy-Induced Nausea and Vomiting. Obstetrics and Gynecology International. 2013;2013:4.

19. Haniadka R, Saldanha E, Sunita V, Palatty PL, Fayad R, Baliga MS. A review of the gastroprotective effects of ginger (Zingiber officinale Roscoe). Food Funct. 2013 Jun;4(6):845-55.

20. Rahmani AH, Shabrmi FM, Aly SM. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. Int J Physiol Pathophysiol Pharmacol. 2014;6(2):125-36.

21. Walstab J, Kruger D, Stark T, Hofmann T, Demir IE, Ceyhan GO, et al. Ginger and its pungent constituents non-competitively inhibit activation of human recombinant and native 5-HT3 receptors of enteric neurons. Neurogastroenterol Motil. 2013 Could;25(5):439-47, e302.

**Walstab is the first study to demonstrate 5-HT_3 antagonism in human 5-HT_3 receptors*

22. Jin Z, Lee G, Kim S, Park CS, Park YS, Jin YH. Ginger and its pungent constituents non-competitively inhibit serotonin currents on visceral afferent neurons. The Korean journal of physiology & pharmacology : official journal of the Korean Physiological Society and the Korean Society of Pharmacology. 2014 Apr;18(2):149-53.

Chapter 4. Ginger - mechanism of action in chemotherapy-induced nausea and vomiting: a review.

Before the publication of this manuscript, there had been no review of the potential mechanisms of action for the active constituents of ginger in regards to CINV. This chapter discusses these various mechanisms and provides recommendations for future research. This manuscript was published in *Critical Reviews in Food Science and Nutrition* (2014 Impact Factor: 5.176) and has been cited twice (07/08/2015; Scopus Citations). In addition, data from this manuscript was presented and received awards at the following conferences:

1. **Marx W**, McCarthy AL, Vitetta L, Ried K, Sali A, McKavanagh D, Isenring E. Ginger - mechanism of action in chemotherapy-induced nausea and vomiting: a review. MASCC/ISOO Symposium (26 – 28 June 2014, Miami, USA).

   Poster presentation – awarded Best Poster Prize
2. **Marx W**, McCarthy AL, Vitetta L, Ried K, Sali A, McKavanagh D, Isenring E. Ginger - mechanism of action in chemotherapy-induced nausea and vomiting: a review. Nutrition Society of Australia (26 – 28 November 2014, Hobart, Australia).

   Poster presentation – awarded Best Student Poster Award

Citation:

Marx W, Ried K, McCarthy AL, Vitetta L, Sali A, McKavanagh D, et al. Ginger-Mechanism of Action in Chemotherapy-induced Nausea and Vomiting: A Review. Crit Rev Food Sci Nutr. 2015:0. Epub 2015/04/08. doi: 10.1080/10408398.2013.865590. PubMed PMID: 25848702

4.1 Abstract

Despite advances in anti-emetic therapy, chemotherapy-induced nausea and vomiting (CINV) still poses a significant burden to patients undergoing chemotherapy. Nausea, in particular, is still highly prevalent in this population. Ginger has been traditionally used as a folk remedy for gastrointestinal complaints and has been suggested as a viable adjuvant treatment for nausea and vomiting in the cancer context. Substantial research has revealed ginger to possess properties that could exert multiple beneficial effects on chemotherapy patients who experience nausea and vomiting. Bioactive compounds within the rhizome of ginger, particularly the gingerol and shogaol class of compounds, interact with several pathways that are directly implicated in CINV in addition to pathways that could play secondary roles by exacerbating symptoms. These properties include 5-HT_3, substance *P* and acetylcholine receptor antagonism; anti-inflammatory properties; and modulation of cellular redox signalling, vasopressin release, gastrointestinal motility, and gastric emptying rate. This review outlines these proposed mechanisms by discussing the results of clinical, *in vitro* and animal studies both within the chemotherapy context and in other relevant fields. The evidence presented in this review indicates that ginger possesses multiple properties that could be beneficial in reducing chemotherapy-induced nausea and vomiting.

4.2 Introduction

Chemotherapy-induced nausea and vomiting (CINV) is a significant burden for patients undergoing anticancer chemotherapy. Nausea and vomiting are rated as two of the most distressing symptoms by chemotherapy patients and have been shown to

significantly and adversely affect quality of life and physical function during treatment.(Carelle et al., 2002; Sun et al., 2005) Ratings of quality of life can be reduced by as much as 20% in patients who experience CINV compared to symptom-free patients.(Lindley et al., 1992) Additionally, CINV is associated with malnutrition and further physical complications such as acid-base imbalance and electrolyte disturbances.(Davidson et al., 2012; Lindley and Hirsch, 1992; Osoba, 2005) All of these issues affect the patients' ability to adhere to, or complete chemotherapy, resulting in a potential concomitant impact on survival outcomes.

Despite significant improvement in the control of CINV through the use of modern anti-emetics such as 5-HT_3 antagonists, corticosteroids and NK1 antagonists, nausea and vomiting still affects up to 60% and 37% of patients undergoing chemotherapy, respectively.(Bloechl-Daum et al., 2006)

Ginger has traditionally been used for centuries as a treatment for gastrointestinal complaints and more recently has been investigated for its use in treating motion sickness, post-operative nausea and vomiting, and morning sickness in clinical studies.(Ernst and Pittler, 2000) A recent systematic review of randomised-controlled trials that investigated the effect of ginger as an adjuvant treatment for CINV found that the literature was equivocal with significant limitations.(Marx et al., 2013)

An array of compounds are bioactive within the rhizome of ginger, such as shogaols, gingerols, zingerone, and paradols.(Baliga et al., 2011) These compounds are typically categorised into two classes: volatile oils and non-volatile pungent compounds.

Both of these classes of compounds are contained within the oleoresin, the collective term for the oil and resin fraction of the rhizome. While the concentration of these compounds varies greatly depending on the country of origin, storage, and preparation of the ginger product, the gingerol and shogaol compounds are likely to be the primary components responsible for ginger's pharmacological effects. These compounds are believed to interact with multiple areas implicated in the development of CINV. Specific properties of these compounds that could be relevant to CINV include 5-HT_3, substance *P* and acetylcholine receptor antagonism; anti-inflammatory properties; and modulation of cellular redox signalling, vasopressin release, gastrointestinal motility, and gastric emptying rate.(Abdel-Aziz et al., 2006; Prakash and Srinivasan, 2010; Wu et al., 2008; Zick et al., 2011) Whereas recent reviews have focused upon the clinical efficacy of ginger, this paper will focus on the potential mechanisms by which ginger could exert anti-CINV effects.

4.3 Physiology of CINV

The physiology of CINV is a complex neural interaction involving central and peripheral stimuli and reactions. While multiple pathways are involved in CINV, this discussion will focus on the primary pathway of CINV (i.e 5-HT_3 and NK1 antagonism) and pathways that could potentially be modulated by ginger (Figure 4-1). The site of the initial trigger of CINV is thought to be within the gastrointestinal tract. Chemotherapy agents interact with enterochromaffin cells, possibly via oxidative stress, resulting in a release of the neurotransmitters serotonin and substance *P*.(Torii et al., 1994a) The released neurotransmitters then interact with receptors located upon the vagus nerve,

which subsequently transmits afferent signals to the chemotherapy receptor zone within the brain via the nucleus tractus solitarius. It is thought that modern 5-HT_3 antagonist medications (e.g. ondansetron) interact with the 5-HT_3 receptors involved in this process, which then mitigates the degree of afferent signalling. Another neurotransmitter, substance *P*, has also been implicated in the generation of CINV by binding to NK_1 receptors located centrally within the brain. Stimuli transmitted using these two neuropeptides, as well as stimuli from other regions of the brain, are processed by the chemoreceptor trigger zone and vomiting centre, which then coordinates the relevant musculature to induce a nausea and/or vomiting response.(Rudd, 2005)

While not directly involved in the generation of CINV, other secondary pathways could exacerbate the experience of nausea and vomiting in this setting. These include the modulation of gastric emptying, increased inflammation, and vestibular and vasopressin-related mechanisms.(Cawley and Benson, 2005; Rudd, 2005; Sharma and Gupta, 1998)

Chemotherapy agents such as cisplatin and methotrexate are known to delay gastric emptying, potentially resulting in gastrointestinal distress due to antral distension.(Sharma and Gupta, 1998) Research related to chemotherapy-induced mucositis has demonstrated that pro-inflammatory signalling pathways, particularly nuclear factor kappa-B (NF-κB), are increased within the gastrointestinal mucosa as a result of chemotherapy-induced cell injury. It has been suggested that this increase in gut inflammation might contribute to the development of CINV, particularly during the delayed phase (≥24 hours after chemotherapy)(Rudd, 2005) which is supported by the

increase in inflammatory cytokines largely occurring between 2-10 days post-chemotherapy.(Cawley and Benson, 2005)

The vestibular system, which is located within the inner ear, is involved in providing a sense of balance. While the vestibular system might not be a primary pathway in the development of CINV, vestibular disturbances are implicated in the exacerbation of CINV. In support of this, the vestibular system is involved in the development of motion sickness, which is a known risk factor for CINV.(Leventhal et al., 1988) Furthermore, scopolamine, a pharmacological treatment for motion sickness, has demonstrated efficacy in reducing CINV when used in conjunction with other anti-emetic medications, but not when used as a stand-alone treatment.(Longo et al., 1982; Meyer et al., 1987) This suggests that the vestibular system plays a secondary role in the development of CINV.

Lastly, it has been suggested that vasopressin (also known as antidiuretic hormone) contributes to the sensation of nausea in chemotherapy patients. Studies have demonstrated that vasopressin is significantly increased in patients experiencing CINV(Fisher et al., 1982; Rudd, 2005) and that the administration of supraphysiological doses of endogenous vasopressin is sufficient to induce nausea in healthy human participants.(Caras et al., 1997) However, other studies do not support this hypothesis. For example, when vasopressin was administered at physiological doses, nausea was not experienced.(Kim et al., 1997) This has lead researchers to suggest that vasopressin could play a modulatory role in the generation of CINV instead.(Rudd, 2005)

4.4 Proposed mechanisms of action

4.4.1 *Interaction with neurotransmitters and vagal afferent signalling*

Results from *in vitro* and animal studies demonstrate that ginger is likely to exert 5-HT_3 antagonistic effects. Yamahara et al.(Yamahara et al., 1989) were the first to demonstrate that whole ginger, as well as 6-, 8- and 10-gingerols, could inhibit 5-HT_3-induced contractions in an isolated guinea pig ileum. Huang et al.(Huang et al., 1991) demonstrated inhibition of 5-HT_3-induced contractions using the ginger compound, galanolactone. However, these two studies have significant limitations.(Abdel-Aziz et al., 2006) Both studies used serotonin to induce contractions, not an agonist that is selective for 5-HT_3 receptors. This allows for the possibility that ginger inhibited the action of serotonin on another receptor, making the exact mechanism of action unclear.(Abdel-Aziz et al., 2006)

Additionally, Huang et al.(Huang et al., 1991) studied galanolactone, a compound only found in Japanese ginger and which therefore cannot be extrapolated to other types of ginger.(Abdel-Aziz et al., 2006; Ravindran and Babu, 2004)

To address these limitations, Abdel-Aziz et al.(Abdel-Aziz et al., 2006) investigated the effect of four major compounds found in ginger, namely 6-, 8- and 10-gingerol and 6-shogaol, on 5-HT_3-mediated contractions in an isolated rat ileum using a selective 5-HT_3 agonist. The results indicated that these compounds significantly inhibited contractions induced by this agonist; however, all four compounds failed to displace the 5-HT_3 receptor antagonist, [^{3}H]GR65630, from binding to the 5-HT_3

receptor. It was therefore concluded that the mechanism of action of ginger, at least in relation to 5-HT_3 pathways, is most likely due to indirect modulation of 5-HT_3 signalling through the binding of an alternative, unidentified site.(Abdel-Aziz et al., 2006) Additionally, the authors reported that these compounds weakly inhibited acetyl-choline and substance *P*-induced contractions, suggesting additional mechanisms for the anti-CINV effects of ginger.

4.4.2 *Modulation of gastrointestinal motility and gastric emptying*

Metoclopramide has been used for decades as an anti-emetic in chemotherapy, partly due to its prokinetic effect on the gastrointestinal system.(Schapira et al., 1990) Research, particularly from *in vitro* studies, suggest that ginger is also likely to affect gastrointestinal motility and gastric emptying.(Hashimoto et al., 2002; Hu et al., 2011; Wu et al., 2008) While gastrointestinal dysmotility could not play a direct role in the generation of CINV, it could play a secondary role by contributing to other gastrointestinal symptoms such as bloating, early satiety, and abdominal pain.

Multiple animal and *in vitro* studies indicate that whole ginger as well as specific compounds within ginger affect gastric emptying rates and gastrointestinal contractions. For example, Hashimoto et al.(Hashimoto et al., 2002) demonstrated that 6-shogaol improved muscle contractions and charcoal-induced transit time in porcine small intestines. Similarly, acetone ginger extract as well as the ginger components, 6-shogoal, 6-. 8-, and 10-gingerol, all enhanced the transport of a charcoal meal in mice.(Yamahara et al., 1990) Furthermore, both an ethanolic and acetone extract of ginger as well as ginger

juice all reversed cisplatin-induced delayed gastric emptying in rats.(Sharma and Gupta, 1998) In contrast, the ginger compounds zingerone and zingerol as well as whole ginger were reported to inhibit colonic motility in rats.(Iwami et al., 2011a; Iwami et al., 2011b) These diverse results indicate that ginger's effects could be a result of the particular concentration of different bioactive compounds, or the synergy between them.

The effect of ginger on gastrointestinal motility in human participants has been investigated in multiple studies; however, the degree to which the results of these studies can be extrapolated to the CINV setting is limited as no study has been conducted with patients undergoing chemotherapy to date. This is likely due to CINV-related anti-emetic research focusing on other pathways (i.e 5-HT_3-mediated CINV) and the burden that such a study could place on patients undergoing chemotherapy; however, relief from symptoms related to gastrointestinal dysmotility could prove to be effective as a secondary measure of CINV management and therefore, future research in the CINV setting is recommended.

To date, six studies have examined the effect of ginger on gastrointestinal motility in varied patient populations, including healthy participants and participants with dyspepsia or admitted to an intensive care unit.(Hu et al., 2011; Micklefield et al., 1999; Phillips et al., 1993; Shariatpanahi et al., 2010; Stewart et al., 1991; Wu et al., 2008) However, the significant differences in methodology employed in these studies makes comparison difficult. Differences included the dosage of ginger, the composition of the test meal used, and the instrument used to measure gastric emptying and motility.

Scintography is the recommended method to evaluate gastric empting.(Abell et al., 2008) However, due to the use of radioactive materials in this technique and the risk attendant on this, alternative methods are preferred.(Wu et al., 2008) While the use of alternative methods might reduce the equipment costs and expertise required, they are not as sensitive and could introduce confounders. For example, in one study of intensive care patients, when gastric emptying was measured by the amount of feeding tolerated over a 48 hour period by participants, ginger improved gastric motility.(Shariatpanahi et al., 2010) However, in an another study that evaluated gastric emptying by a similarly indirect method (the measurement of paracetamol absorption), Phillips et al.(Phillips et al., 1993) found 1g of ginger had no effect on gastric emptying. The indirect measures used in these two studies provided a lower level of precision. The results could also be influenced by other factors, such as the nutrient density of the test meal, its fluid and macronutrient content and its total volume. All of these factors can influence the rate of gastric emptying; hence, a nutrient-dense test meal is critical when measuring rates of gastric emptying.(Wu et al., 2008) Because no test meal was used in this study, significant delays in gastric emptying would not be expected. A similarly non-nutrient-dense test meal was used in a study of the effect of 500mg of ginger on gastric emptying rates.(Stewart et al., 1991) The failure of this study to demonstrate efficacy in relation to ginger could be a result of the 75kcal solution used as the test meal, which could have been insufficient to induce an effect.(Stewart et al., 1991)

Wu et al.(Wu et al., 2008) and Hu et al.(Hu et al., 2011) addressed many of these limitations by using a dose of 1.2g of ginger and a test meal with a relatively high caloric

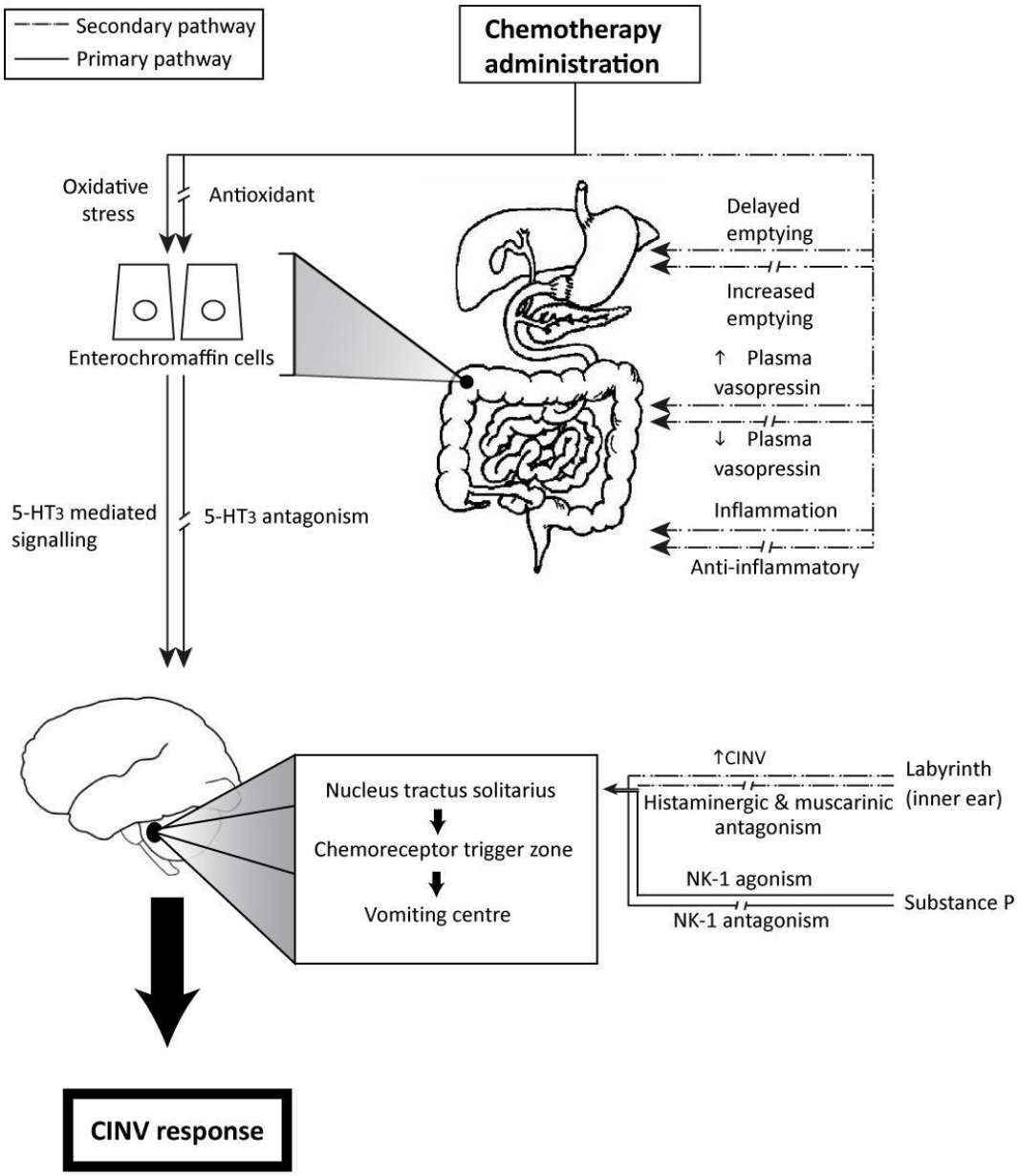
content (118kcal in both studies). The two studies found that ginger was effective at reducing gastric emptying rates in both healthy and dyspeptic participants. A smaller dose of ginger (200mg) has also demonstrated effectiveness in increasing gastrointestinal motility in healthy volunteers.(Micklefield et al., 1999)

In summary, animal studies as well as most human studies conducted to date (66%) suggest ginger modulates the rate of gastric emptying and gastrointestinal motility. However, no studies so far have investigated the effect in participants undergoing chemotherapy and therefore, the applicability of these results to the chemotherapy setting is currently unclear.

4.4.3 *Anti-oxidant properties*

Oxidative stress, defined as an over production of reactive oxygen species, has been reported to be linked to the etiology of the emetic reflex. One of the initial steps in the generation of CINV is believed to be the generation of free radicals by chemotherapy agents within the gastrointestinal tract which in turn leads to the release

Figure 4-1. Proposed anti-CINV mechanisms of action of ginger



of neurotransmitters from enterochromaffin cells.(Torii et al., 1994b) This notion has led to investigations of the antioxidant activity of ginger. *In vitro* experiments have demonstrated the antioxidant kinetic behaviour of isolated compounds extracted from the

dried rhizomes of ginger, subjected to a 1,1-diphenyl-2-picrylhydrazyl radical scavenging reaction.(Masuda et al., 2004)

However, there are no clear human clinical trials or animal experiments that demonstrate that ginger extracts might modulate CINV via an antioxidant effect. Given that the oxidative stress/antioxidant theory of cellular metabolism has been challenged,(Linnane et al., 2007) an alternative plausible biochemical explanation for ginger's effect on CINV is the rebalancing of the disrupted cellular oxido-reductase mechanism that often accompanies chemotherapy treatments.(Linnane et al., 2007)

4.4.4 *Anti-inflammatory properties*

During chemotherapy, cell injury caused within the gastrointestinal tract (GIT) results in the release of multiple inflammatory factors including cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), and nuclear factor kappa-B (NF-kB). The end result of this pathway is continued tissue damage and potentially mucositis along the length of the GIT.(Sultani et al., 2012) It has been suggested that inflammation and cell injury could be particularly involved in the delayed phase of CINV.(Hesketh, 2005)

In vitro research has found that multiple ginger compounds are able to elicit an anti-inflammatory effect through a number of pathways including the inhibition of NF-kB, COX enzymes, and 5-lipoxogenase.(van Breemen et al., 2011) Ginger compounds have also demonstrated an anti-inflammatory effect in murine and rat models, with these effects replicated in human clinical trials.(Ojewole, 2006; Zick et al., 2011) For example, 28 days of ginger supplementation (2g) in humans modulated eicosanoid synthesis in the

colonic mucosa by lowering prostaglandin-2 levels in healthy participants(Zick et al., 2011) and COX-1 in participants who were at risk of colon cancer.(Jiang et al., 2012) Additionally, a review that included 8 clinical trials in this field concluded that while there is a paucity of well-designed trials, there is tentative evidence that ginger possesses anti-inflammatory properties in the treatment of pain related to osteoarthritis, dysmenorrhea, and exercise.(Terry et al., 2011)

In summary, while these studies did not directly measure the effect of ginger on inflammation during chemotherapy, the current literature indicates that ginger is likely to modulate inflammation in the gut and this could contribute to ginger's anti-CINV effects.

4.4.5 *Vestibular interactions*

Acetylcholine and histamine are two neurotransmitters involved in the development of motion sickness. *In vitro* studies demonstrate that ginger compounds have antagonistic properties to both muscarinic and histaminergic receptors and therefore, represent a potential pathway by which ginger could interact with the vestibular system.(Abdel-Aziz et al., 2006) Clinical trials have largely confirmed this effect in clinical or experimentally-induced motion sickness. Eight trials were identified in our review, of which five reported ginger to be either superior to placebo or equal to standard anti-motion sickness medications. (Grontved et al., 1988; Grontved and Hentzer, 1986; Holtmann et al., 1989; Lien et al., 2003; Mowrey and Clayson, 1982; Schmid et al., 1994; Stewart et al., 1991; Wood et al., 1988) Therefore, it is likely that ginger is able to interact

with signalling involved in the vestibular system and could potentially modulate CINV symptoms.

4.4.6 *Modulation of vasopressin*

Ginger is known to reduce plasma vasopressin in adults exposed to experimentally-induced motion sickness; however, when endogenous vasopressin was injected, ginger was ineffective in preventing nausea.(Lien et al., 2003) This suggests that ginger exerts an indirect action on vasopressin release. However, to date there is only one study measuring ginger's effect on vasopressin. Future studies are required to confirm these effects in the chemotherapy setting. Furthermore, the exact role of vasopressin in CINV needs to be elucidated before this can be considered a clinically-relevant mechanism.

4.5 Conclusion

CINV is a significant burden experienced by many oncology patients. While the control of overt vomiting has advanced, it is still prevalent and nausea remains stubbornly problematic for numerous chemotherapy patients. Ginger contains a wide array of bioactive compounds that can potentially act on multiple pathways involved in the physiology of CINV (Figure 4-1). These pathways include the modulation of relevant neuropeptides, vasopressin release and gastrointestinal motility as well as redox and anti-inflammatory signalling. The clinical evidence for its use in the treatment in CINV is currently equivocal;(Marx et al., 2013) however, the data presented in this paper demonstrate an array of viable mechanisms of action and provide a sound foundation for

continued research in this area. Of primary importance is the need for future trials to investigate these beneficial properties in the chemotherapy setting.

4.6 References

1 Abdel-Aziz, H., Windeck, T., Ploch, M., and Verspohl, E. J. (2006). Mode of action of gingerols and shogaols on 5-HT3 receptors: Binding studies, cation uptake by the receptor channel and contraction of isolated guinea-pig ileum. *European Journal of Pharmacology*. 530: 136-143.

2 Abell, T. L., Camilleri, M., Donohoe, K., Hasler, W. L., Lin, H. C., Maurer, A. H., McCallum, R. W., Nowak, T., Nusynowitz, M. L., Parkman, H. P., Shreve, P., Szarka, L. A., Snape, W. J., Jr., and Ziessman, H. A. (2008). Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. *Am J Gastroenterol*. 103: 753-763.

3 Baliga, M. S., Haniadka, R., Pereira, M. M., D'Souza, J. J., Pallaty, P. L., Bhat, H. P., and Popuri, S. (2011). Update on the chemopreventive effects of ginger and its phytochemicals. *Crit Rev Food Sci Nutr*. 51: 499-523.

4 Bloechl-Daum, B., Deuson, R. R., Mavros, P., Hansen, M., and Herrstedt, J. (2006). Delayed nausea and vomiting continue to reduce patients' quality of life after highly and moderately emetogenic chemotherapy despite antiemetic treatment. *J Clin Oncol*. 24: 4472-4478.

5 Caras, S. D., Soykan, I., Beverly, V., Lin, Z., and McCallum, R. W. (1997). The effect of intravenous vasopressin on gastric myoelectrical activity in human subjects. *Neurogastroenterology & Motility*. 9: 151-156.

6 Carelle, N., Piotto, E., Bellanger, A., Germanaud, J., Thuillier, A., and Khayat, D. (2002). Changing patient perceptions of the side effects of cancer chemotherapy. *Cancer*. 95: 155-163.

7 Cawley, M. M., and Benson, L. M. (2005). Current trends in managing oral mucositis. *Clin J Oncol Nurs*. 9: 584-592.

8 Davidson, W., Teleni, L., Muller, J., Ferguson, M., McCarthy, A. L., Vick, J., and Isenring, E. (2012). Malnutrition and chemotherapy-induced nausea and vomiting: implications for practice. *Oncol Nurs Forum*. 39: E340-345.

9 Ernst, E., and Pittler, M. H. (2000). Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials. *British Journal of Anaesthesia*. 84: 367-371.

10 Fisher, R. D., Rentschler, R. E., Nelson, J. C., Godfrey, T. E., and Wilbur, D. W. (1982). Elevation of plasma antidiuretic hormones (ADH) associated with chemotherapy-induced emesis in man. *Cancer Treat Rep*. 66: 25-29.

11 Grontved, A., Brask, T., Kambskard, J., and Hentzer, E. (1988). Ginger root against seasickness. A controlled trial on the open sea. *Acta Otolaryngol*. 105: 45-49.

12 Grontved, A., and Hentzer, E. (1986). Vertigo-reducing effect of ginger root. A controlled clinical study. *ORL J Otorhinolaryngol Relat Spec*. 48: 282-286.

13 Hashimoto, K., Satoh, K., Murata, P., Makino, B., Sakakibara, I., Kase, Y., Ishige, A., Higuchi, M., and Sasaki, H. (2002). Component of Zingiber officinale that improves the enhancement of small intestinal transport. *Planta Med*. 68: 936-939.

14 Hesketh, P. J. (2005). Management of Nausea and Vomiting in Cancer Treatment: Introduction, Scope of the Problem. In: Management of Nausea and Vomiting in Cancer and Cancer Treatment, pp. 1-15. Hesketh, P. J. (Ed.), Jones and Bartlett, Sudbury, MA.

15 Holtmann, S., Clarke, A. H., Scherer, H., and Hohn, M. (1989). The anti-motion sickness mechanism of ginger. A comparative study with placebo and dimenhydrinate. *Acta Otolaryngol*. 108: 168-174.

16 Hu, M. L., Rayner, C. K., Wu, K. L., Chuah, S. K., Tai, W. C., Chou, Y. P., Chiu, Y. C., Chiu, K. W., and Hu, T. H. (2011). Effect of ginger on gastric motility and symptoms of functional dyspepsia. *World J Gastroenterol*. 17: 105-110.

17 Huang, Q. R., Iwamoto, M., Aoki, S., Tanaka, N., Tajima, K., Yamahara, J., Takaishi, Y., Yoshida, M., Tomimatsu, T., and Tamai, Y. (1991). Anti-5-hydroxytryptamine3 effect of galanolactone, diterpenoid isolated from ginger. *Chem Pharm Bull (Tokyo)*. 39: 397-399.

18 Iwami, M., Shiina, T., Hirayama, H., Shima, T., Takewaki, T., and Shimizu, Y. (2011a). Inhibitory effects of zingerone, a pungent component of Zingiber officinale Roscoe, on colonic motility in rats. *J Nat Med*. 65: 89-94.

19 Iwami, M., Shiina, T., Hirayama, H., and Shimizu, Y. (2011b). Intraluminal administration of zingerol, a non-pungent analogue of zingerone, inhibits colonic motility in rats. *Biomed Res*. 32: 181-185.

20 Jiang, Y., Turgeon, D. K., Wright, B. D., Sidahmed, E., Ruffin, M. T., Brenner, D. E., Sen, A., and Zick, S. M. (2012). Effect of ginger root on cyclooxygenase-1 and

15-hydroxyprostaglandin dehydrogenase expression in colonic mucosa of humans at normal and increased risk for colorectal cancer. *Eur J Cancer Prev*.

21 Kim, M. S., Chey, W. D., Owyang, C., and Hasler, W. L. (1997). Role of plasma vasopressin as a mediator of nausea and gastric slow wave dysrhythmias in motion sickness. *Am J Physiol*. 272: G853-862.

22 Leventhal, H., Easterling, D. V., Nerenz, D. R., and Love, R. R. (1988). The role of motion sickness in predicting anticipatory nausea. *J Behav Med*. 11: 117-130.

23 Lien, H. C., Sun, W. M., Chen, Y. H., Kim, H., Hasler, W., and Owyang, C. (2003). Effects of ginger on motion sickness and gastric slow-wave dysrhythmias induced by circular vection. *Am J Physiol Gastrointest Liver Physiol*. 284: G481-489.

24 Lindley, C. M., and Hirsch, J. D. (1992). Nausea and vomiting and cancer patients' quality of life: a discussion of Professor Selby's paper. *Br J Cancer Suppl*. 19: S26-29.

25 Lindley, C. M., Hirsch, J. D., O'Neill, C. V., Transau, M. C., Gilbert, C. S., and Osterhaus, J. T. (1992). Quality of life consequences of chemotherapy-induced emesis. *Qual Life Res*. 1: 331-340.

26 Linnane, A. W., Kios, M., and Vitetta, L. (2007). Healthy aging: regulation of the metabolome by cellular redox modulation and prooxidant signaling systems: the essential roles of superoxide anion and hydrogen peroxide. *Biogerontology*. 8: 445-467.

27 Longo, D. L., Wesley, M., Howser, D., Hubbard, S. M., Anderson, T., and Young, R. C. (1982). Results of a randomized double-blind crossover trial of scopolamine

versus placebo administered by transdermal patch for the control of cisplatin-induced emesis. *Cancer Treat Rep*. 66: 1975-1976.

28 Marx, W. M., Teleni, L., McCarthy, A. L., Vitetta, L., McKavanagh, D., Thomson, D., and Isenring, E. (2013). Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. *Nutrition Reviews*. 71: 245-254.

29 Masuda, Y., Kikuzaki, H., Hisamoto, M., and Nakatani, N. (2004). Antioxidant properties of gingerol related compounds from ginger. *Biofactors*. 21: 293-296.

30 Meyer, B. R., O'Mara, V., and Reidenberg, M. M. (1987). A controlled clinical trial of the addition of transdermal scopolamine to a standard metoclopramide and dexamethasone antiemetic regimen. *J Clin Oncol*. 5: 1994-1997.

31 Micklefield, G. H., Redeker, Y., Meister, V., Jung, O., Greving, I., and Could, B. (1999). Effects of ginger on gastroduodenal motility. *Int J Clin Pharmacol Ther*. 37: 341-346.

32 Mowrey, D. B., and Clayson, D. E. (1982). Motion sickness, ginger, and psychophysics. *Lancet*. 1: 655-657.

33 Ojewole, J. A. (2006). Analgesic, antiinflammatory and hypoglycaemic effects of ethanol extract of Zingiber officinale (Roscoe) rhizomes (Zingiberaceae) in mice and rats. *Phytother Res*. 20: 764-772.

34 Osoba, D. (2005). Impact of Nausea and Vomiting on Health-Related Quality of Life. In: Management of Nausea and Vomiting in Cancer and Cancer Treatment, pp. 99-118. Hesketh, P. J. (Ed.), Jones and Bartlett, Sudbury, MA.

35 Phillips, S., Hutchinson, S., and Ruggier, R. (1993). Zingiber officinale does not affect gastric emptying rate. A randomised, placebo-controlled, crossover trial. *Anaesthesia*. 48: 393-395.

36 Prakash, U. N., and Srinivasan, K. (2010). Gastrointestinal protective effect of dietary spices during ethanol-induced oxidant stress in experimental rats. *Appl Physiol Nutr Metab*. 35: 134-141.

37 Ravindran, P. N., and Babu, K. N. (2004). In: Ginger: The Genus Zingiber, p. 86. Taylor & Francis.

38 Rudd, J. A., Andrews, P.L.R (2005). Mechanisms of acute, delayed, and anticipatory emesis induced by anticancer therapies. In: Management of Nausea and Vomiting in Cancer and Cancer Treatment, pp. 15-65. Hesketh, P. J. (Ed.), Jones and Bartlett, Sudbury, MA.

39 Schapira, M., Henrion, J., and Heller, F. R. (1990). The current status of gastric prokinetic drugs. *Acta Gastroenterol Belg*. 53: 446-457.

40 Schmid, R., Schick, T., Steffen, R., Tschopp, A., and Wilk, T. (1994). Comparison of Seven Commonly Used Agents for Prophylaxis of Seasickness. *J Travel Med*. 1: 203-206.

41 Shariatpanahi, Z. V., Taleban, F. A., Mokhtari, M., and Shahbazi, S. (2010). Ginger extract reduces delayed gastric emptying and nosocomial pneumonia in adult respiratory distress syndrome patients hospitalized in an intensive care unit. *J Crit Care*. 25: 647-650.

42 Sharma, S. S., and Gupta, Y. K. (1998). Reversal of cisplatin-induced delay in gastric emptying in rats by ginger (Zingiber officinale). *J Ethnopharmacol*. 62: 49-55.

43 Stewart, J. J., Wood, M. J., Wood, C. D., and Mims, M. E. (1991). Effects of ginger on motion sickness susceptibility and gastric function. *Pharmacology*. 42: 111-120.

44 Sultani, M., Stringer, A. M., Bowen, J. M., and Gibson, R. J. (2012). Anti-Inflammatory Cytokines: Important Immunoregulatory Factors Contributing to Chemotherapy-Induced Gastrointestinal Mucositis. *Chemotherapy Research and Practice*. 2012: 11.

45 Sun, C. C., Bodurka, D. C., Weaver, C. B., Rasu, R., Wolf, J. K., Bevers, M. W., Smith, J. A., Wharton, J. T., and Rubenstein, E. B. (2005). Rankings and symptom assessments of side effects from chemotherapy: insights from experienced patients with ovarian cancer. *Support Care Cancer*. 13: 219-227.

46 Terry, R., Posadzki, P., Watson, L. K., and Ernst, E. (2011). The use of ginger (Zingiber officinale) for the treatment of pain: a systematic review of clinical trials. *Pain Med*. 12: 1808-1818.

47 Torii, Y., Saito, H., and Matsuki, N. (1994a). Induction of emesis in Suncus murinus by pyrogallol, a generator of free radicals. *British Journal of Pharmacology*. 111: 431-434.

48 Torii, Y., Saito, H., and Matsuki, N. (1994b). Induction of emesis in Suncus murinus by pyrogallol, a generator of free radicals. *Br J Pharmacol*. 111: 431-434.

49 van Breemen, R. B., Tao, Y., and Li, W. (2011). Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale). *Fitoterapia*. 82: 38-43.

50 Wood, C. D., Manno, J. E., Wood, M. J., Manno, B. R., and Mims, M. E. (1988). Comparison of efficacy of ginger with various antimotion sickness drugs. *Clin Res Pr Drug Regul Aff*. 6: 129-136.

51 Wu, K. L., Rayner, C. K., Chuah, S. K., Changchien, C. S., Lu, S. N., Chiu, Y. C., Chiu, K. W., and Lee, C. M. (2008). Effects of ginger on gastric emptying and motility in healthy humans. *Eur J Gastroenterol Hepatol*. 20: 436-440.

52 Yamahara, J., Huang, Q. R., Li, Y. H., Xu, L., and Fujimura, H. (1990). Gastrointestinal motility enhancing effect of ginger and its active constituents. *Chem Pharm Bull (Tokyo)*. 38: 430-431.

53 Yamahara, J., Rong, H. Q., Iwamoto, M., Kobayashi, G., Matsuda, H., and Fujimura, H. (1989). Active components of ginger exhibiting anti-serotonergic action. *Phytotherapy Research*. 3: 70-71.

54 Zick, S. M., Turgeon, D. K., Vareed, S. K., Ruffin, M. T., Litzinger, A. J., Wright, B. D., Alrawi, S., Normolle, D. P., Djuric, Z., and Brenner, D. E. (2011). Phase II study of the effects of ginger root extract on eicosanoids in colon mucosa in people at normal risk for colorectal cancer. *Cancer Prev Res (Phila)*. 4: 1929-1937.

Chapter 5. The effect of ginger (Zingiber officinale) on platelet aggregation: a systematic literature review.

The potential effect of ginger on platelet aggregation is a widely-cited concern both within the published literature and by clinicians; however, there had been no systematic appraisal of the evidence prior to this manuscript. In this systematic review of the literature, all existing clinical and observational data regarding the potential effect of ginger on platelet aggregation were evaluated. This abstract was presented at the following conference:

Marx W, McKavanagh D, McCarthy AL, Bird R, Chan A, Ried K, Isenring E. The effect of ginger (*Zingiber officinale*) on platelet aggregation: a systematic literature review. MASCC/ISOO Symposium (25 – 27 June 2015, Copenhagen, Denmark).

Citation:

Marx W, McKavanagh D, McCarthy AL, et al. The Effect of Ginger (Zingiber officinale) on Platelet Aggregation: A Systematic Literature Review. PLoS ONE. 2015;10:e0141119. (Impact factor: Impact Factor: 3.2).

5.1 Abstract

Background: The potential effect of ginger on platelet aggregation is a widely-cited concern both within the published literature and to clinicians; however, there has been no systematic appraisal of the evidence to date.

Methods: Using the PRISMA guidelines, we systematically reviewed the results of clinical and observational trials regarding the effect of ginger on platelet aggregation in adults compared to either placebo or baseline data. Studies included in this review stipulated the independent variable was a ginger preparation or isolated ginger compound, and used measures of platelet aggregation as the primary outcome.

Results: Ten studies were included, comprising eight clinical trials and two observational studies. Of the eight clinical trials, four reported that ginger reduced platelet aggregation, while the remaining four reported no effect. The two observational studies also reported mixed findings.

Discussion: Many of the studies appraised for this review had moderate risks of bias. Methodology varied considerably between studies, notably the timeframe studied, dose of ginger used, and the characteristics of subjects recruited (e.g. healthy vs. patients with chronic diseases).

Conclusion: The evidence that ginger affects platelet aggregation and coagulation is equivocal and further study is needed to definitively address this question.

Key words: ginger, platelet, coagulation, thrombocytopenia

5.2 Introduction

There is increasing evidence that ginger and its constituents might exert meaningful anti-nausea effects during cancer chemotherapy. Our recent systematic review of the literature found preliminary evidence that supported its use as an adjuvant anti-nausea drug to standard anti-emetics in the chemotherapy setting.[1] Concerns over potential "off target" antiplatelet effects, however, could limit the application of ginger in oncology patients, who frequently experience thrombocytopenia due to myelosuppression.

The ginger rhizome has been used in traditional systems of medicine for centuries and more recently, its potentially medicinal properties have been empirically studied.[2] Current research suggests that the active constituents of ginger, namely the gingerol and shogaol classes of compounds, might exert several beneficial effects including anti-inflammatory, antioxidant, and cholesterol lowering properties.[2] In addition, ginger is a promising treatment for nausea associated with a variety of stimuli including post-operative nausea and vomiting, motion sickness, morning sickness, and chemotherapy-induced nausea and vomiting.[1, 3-5]

While the safety profile of ginger supplementation requires further investigation, previous clinical trials report few side-effects, mostly minor in nature (e.g. mild nausea, heartburn).[1] Of these reported side effects, potentially the most significant is an antiplatelet effect. Two published case-studies reported adverse symptoms and abnormal platelet aggregation that was temporally related to recent ingestion of ginger products.[6, 7] In addition, several animal and *in vitro* studies have reported ginger as well as

individual ginger compounds to have an effect on platelet aggregation.[8-10] While this action could be beneficial in vascular diseases, it could potentiate bleeding risk in conditions such as thrombocytopenia or pre-existing platelet dysfunction. This is particularly relevant in the chemotherapy setting, where therapy-induced thrombocytopenia is associated with treatment delays, dose reductions, and bleeding events.[11]

To the authors knowledge, Srivastava et al.[8] were the first group to investigate the effect of ginger on platelet aggregation by using four ginger extracts, produced using different solvents (aqueous, n-hexane, chloroform, and ethyl acetate). They reported that ginger inhibited platelet aggregation using arachidonic acid (AA), epinephrine, adenosine diphosphate (ADP), and collagen as agonists. Others have corroborated this, reporting that certain ginger compounds inhibit *in vitro* platelet aggregation when using a variety of agonists (AA, collagen, platelet activating factor, and thrombin).[12, 13] This reduction in platelet aggregation was most potent when AA was used as the agonist, requiring lower concentrations to cause inhibition when compared to the other agonists.[9, 12]

While few studies investigating the effect of ginger and its compounds on the clotting cascade have been undertaken, a considerable amount of *in vitro* research suggests that ginger compounds interact with AA-derived eicosanoid and thromboxane synthesis.[14-18] The AA cascade can produce the eicosanoids involved in inflammation (i.e. prostaglandin E2) as well as thromboxane, which is amongst the many agonists of

platelet aggregation. Numerous studies indicate that ginger extract and particular ginger compounds inhibit products specific to the cyclooxygenase pathway, including a reduction in thromboxane B_2 (TxB_2) production,[19] prostaglandin formation (PGF2a, PGE_2, and PGD_2),[8, 15] and cyclooxygenase enzyme activity.[16, 18] These same compounds also interact with the lipoxygenase pathway, including reductions in 5-lipoxygenase enzyme activity.[14] Finally, ginger compounds might also inhibit the activity of phospholipase A_2, which suggests that ginger exerts its anti-platelet aggregating as well as its potential anti-inflammatory actions through interaction with one of the initial steps in this pathway.[20]

Due to the observed *in vitro* effects of ginger on the AA cascade, excessive bleeding and interactions with platelet therapy during cancer chemotherapy are of clinical concern. While the results of *in vitro* studies are consistent, these results are not always translatable to the complex human system. Clinical and observational data, however, provide a reasonable indication of the potential human response. There is a growing body of clinical and epidemiological literature in this area, although no systematic appraisal of the relevant literature has been undertaken to date. In this paper, we summarise and discuss the findings of clinical and observational studies regarding the effect of ginger, compared to placebo or baseline, on platelet aggregation in multiple participant populations.

5.3 Methodology

5.3.1 *Data Sources and Searches*

Using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines,[21] a systematic search of the literature was conducted using the following databases: MEDLINE, CiNAHL, Embase, and Cochrane Library. The reference lists of retrieved papers were also searched for additional manuscripts. Search terms were not limited by a specific timeframe; rather, all search queries were from the date of the journal's inception to May 2014. Search terms were broad so as to ensure all relevant manuscripts were captured.

5.3.2 *Study Selection*

The search terms used were "ginger AND (platelet OR thrombo* OR clot* OR bleed OR "adverse effects" OR "side effects" OR haemorrhage)". Studies included in this review 1) were written in English 2) stipulated the independent variable was a ginger preparation or isolated ginger compound, and 3) used measures of platelet aggregation as the primary outcome.

5.3.3 *Data Extraction and Quality Assessment*

Extracted data included: participant demographic (e.g age, gender, reported comorbidities), type of ginger intervention (e.g dosage, timing, form of ginger), study design characteristics (e.g. sample size, risk of bias, type of study, study length), and reported outcomes (e.g measures of platelet aggregation, adverse events, dropout rates).

All clinical studies were individually rated for evidence level by author WM using the National Health and Medical Research Council Hierarchy of Evidence guidelines (IV-I, with I being the strongest level of evidence).[22] They were also independently assessed for bias, by two authors (WM and DM) using the Cochrane Handbook for Systematic Reviews of Interventions checklist.[23] Where insufficient information was included in the manuscript to assess particular forms of bias, further information was sought via correspondence with the study authors. Blinding is unlikely to affect the results of the clinical biomarkers measured in these studies, hence trials that were not blinded were rated in the review as low-risk for detection and performance included bias. In addition, due to the small number of trials in this area, no study was excluded based on its risk of bias.

5.3.4 *Data Synthesis and Analysis*

A statistically significant ($P \leq 0.05$) result was considered evidence of an effect. Relevant study details were retrieved from their respective manuscript using a standardised form. Forest plot and meta-analysis was intended; however, due to the heterogeneity of the studies included in this review, these analyses were found to be unfeasible.

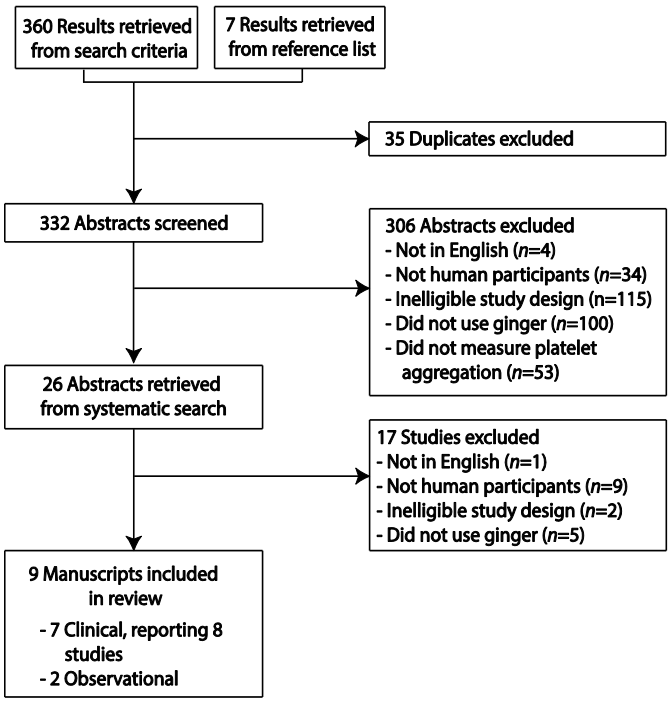
5.4 Results

A total of 367 papers were identified (Figure 5-1). After assessment of study abstracts and the removal of duplicates 26 abstracts were retrieved for further examination. Seventeen were subsequently excluded, resulting in 9 manuscripts included in the final review.

5.4.1 *Clinical trials*

Seven manuscripts reporting the effect of ginger on platelet aggregation in human participants using a clinical trial design were retrieved (Table 5-1). Of the seven manuscripts, one described two separate trials, resulting in a total of eight clinical trials included in this review.[24]

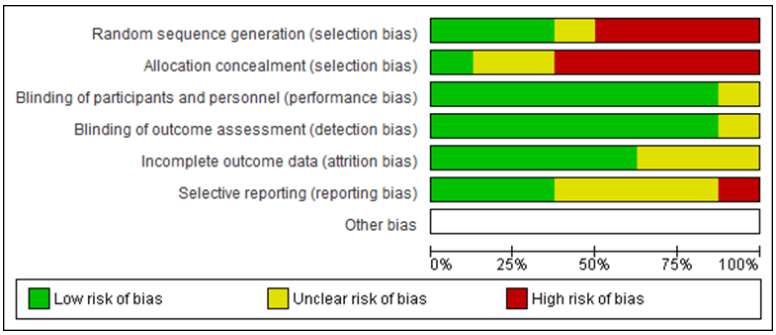
Figure 5-1 PRISMA Study flow diagram



The methodology varied considerably between trials. Half of the studies used a cross-over design[25-28] while three used a parallel design[19, 24] and one was a single arm study.[19] Most of the studies (7/8) had elements of robust study design such as placebo controls, randomisation and double-blinding. However, few studies incorporated all of these elements, with only two studies featuring both randomisation and double-blinding procedures. For example, Jiang et al.[26] used a randomised cross-over design

that was also open-label (Table 5-1). Despite this, the assessment of bias determined the majority of studies were relatively low-risk in terms of performance, detection, and attrition bias while a high risk of random sequence generation and allocation concealment bias was detected (Figure 5-2 Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.).

Figure 5-2 Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.



The average sample size was small. Seven of the eight studies ranged from 7-36 participants[19, 25-29] with one study comprising 60 participants.[24] The duration of each study varied considerably, ranging from one day to three months. Six of the eight studies included healthy participants,[19, 25-27, 29] two studies included patients with confirmed myocardial infarction and one study included hypertensive patients as well as healthy participants.[24, 28] Most studies required participants to consume only ginger, either as a supplement or as a food preparation, while three studies measured the effect of ginger in combination with various medications and food products including nifedipine,[28] warfarin,[26] custard,[30] and a high-calorie diet.[29]

Table 5-1 Extraction table of reviewed clinical trials.

Author/Date	Study design	Time points	Population	Intervention	Outcome	Results	Country	Level of evidence	Comment
Bordia et al. 1997	Placebo-controlled trial	Total study period: 3 months. Outcomes measured at: baseline, 1.5 months and 3 months.	Patients with confirmed myocardial infarction N=60	Dose: 4g per day Unstandardized capsules	Platelet aggregation - Agonist(s): ADP and Epi - Method (Device, if reported): Turbidimetric Fibrinogen Fibrinolytic activity	Ginger had no significant effect on both measures of aggregation	India	III-1*	Ginger had no significant effect on blood lipids or blood sugar. Results relating to fenugreek excluded from table. No mention of randomisation P value not reported

Bordia et al. 1997	Placebo-controlled trial	Total study period: One day Outcomes measured at: baseline, 4 hours post-consumption	Patients with confirmed myocardial infarction N= 20	Dose: 10g single dose Unstandardized capsules	Platelet aggregation - Agonist(s): ADP and Epi - Method (Device, if reported): Turbidimetric	Reduction of both measures of platelet aggregation when compared to placebo ($p<0.05$).	India	III-1	This study was detailed in same manuscript as above.
Janssen et al. 1996	Randomised, placebo-controlled cross-over trial	Total study period: 6 weeks (3x2 weeks) Outcomes measured at day 12 and 14 of each study period.	Healthy volunteers Age: 22±3 N= 18	Dose: 15g raw & 40g cooked ginger placebo, once per day. Contained within 125g custard	Thromboxane B2 production (Payton Aggregation Module)	Both types of ginger had no significant effect on maximum thromboxane B2 production ($p=0.616$)	Netherlands	II	

Jiang et al. 2004	Randomized, open label, three-way cross-over trial	Total study period: 3x13 days, 14 days washout period between each study period. Outcomes measured at multiple time points, starting 2 days pre-warfarin consumption to 7 days post-consumption	Healthy male volunteers Age: 20–36 N= 12	Dose: 3.6g (3x 0.4g, thrice per day) Unstandardized capsules Consumed with 25 mg dose of rac-warfarin, consumed once per study period.	Platelet aggregation - Agonist(s): AA - Method (Device, if reported): Turbidimetric (Chrono-log) INR Plasma warfarin enantiomer protein binding & warfarin enantiomer concentrations Urinary S-7-hydroxywarfarin	No significant changes in any outcome	Australia	III-1	No placebo group was included in study Results relating to participants receiving ginkgo supplementation were excluded from table. P value not reported

Lumb. 1994	Randomised, double-blinded placebo-controlled cross-over trial	Total study period: 2x1 day, at least 14 days washout period. Outcomes measured immediately before, 3h, and 24h post consumption of ginger	Healthy male volunteers N= 8	Dose: 2g (4x500mg) dried ginger per day Unstandardized capsules	Platelet aggregation - Agonist(s): AA, collagen, ristocetin, ADP - Method (Device, if reported): Electrical impedance (Chrono-log) Bleeding time Platelet count Thromboelastography	No significant changes in any outcome at any time point.	UK	II	
Srivastava 1989	Open-label single-arm trial	Total study period: 7 days Outcomes measured at baseline and 7 days post-consumption	Health female volunteers N= 7	Dose: 5g raw ginger per day	Platelet thromboxane B2 production	Ginger consumption resulted in a 37% inhibition of thromboxane B2 production ($p<0.01$) .	Denmark	III-3	Results relating to onion group excluded from table.

Verma et al. 1993	Randomised placebo-controlled trial	Total study period: 14 days, high-calorie diet for first 7 days, high-calorie diet and ginger/placebo consumed for next 7 days. Outcomes measured at baseline, 7, and 14 days	Health male volunteers N= 20	Dose: 5g (4x625mg, twice per day) dry ginger powder Unstandardized capsules Consumed with 100g (2x50g) butter, 2 cups of milk, 8 slices of bread.	Platelet aggregation - Agonist(s): ADP and Epi - Method (Device, if reported): turbidimetric (ELVI-840)	Ginger significantly reduced platelet aggregation using both agonists when compared to placebo group ($p<0.001$).	India	II	Platelet aggregation reduced close to baseline but did not decrease further.

Young et al. 2006	Cross-over trial	Total study period: 72 days, 4x washout period of 7-10 days, 5x7 days intervention consumed Outcomes measured at baseline and 7 days post-consumption for each intervention	Healthy & Hypertensive volunteers N= 10 for each group	Dose: 1g dried ginger per day Either alone or in combination with 10mg nifedipine	Platelet aggregation - Agonist(s): ADP, Epi, collagen - Method (Device, if reported): Turbidimetric (Chronolog 560)	Ginger combined with nifedipine resulted in a significant decrease in platelet aggregation ($p<0.001$). Ginger alone had no significant effect.	Taiwan	III-2	No placebo group Unclear if participants were blinded

Abbreviations: AA, arachidonic acid; ADP, Adenosine Diphosphate; Epi, epinephrine; INR, International Normalised Ratio; TxB_2, Thromboxane B_2;. * Indicates some study details were missing and that scoring was based on details available.

In terms of the ginger preparation used, seven of the eight studies tested a dose of 3.6g to 5g, while one cross-over study investigated larger doses of ginger with each participant receiving either 10g or 40g per day.[30] Most studies delivered ginger at either one time point or once per day, depending on the trial timeframe; however, Jiang et al.[26] and Verma et al.[29] delivered ginger thrice and twice per day, respectively. All studies used an unstandardized ginger preparation, either dried, cooked or raw ginger, delivered in an unprocessed form, within capsules, or mixed into a medium (i.e. custard).

Measures of platelet aggregation varied between studies. The majority (6/8) used light transmittance aggregometry or impedance aggregometry,[24, 26-29] while two studies assessed thromboxane B_2 production.[19, 25] Three studies also recorded multiple additional outcomes including INR,[26] fibrinogen and fibrinolytic activity,[24] bleeding time, thromboelastography and platelet count.[27] Of the six that used aggregometry, there was a mix of agonists used with ADP (5/6) and epinephrine (4/6) being the most common. Three studies also used one or more of the following agonists: collagen, AA, or ristocetin.[26-28]

The reported effect of ginger on platelet function were equivocal. Two studies reported inhibition of platelet aggregation.[24, 29] The first study found that ginger significantly inhibited platelet aggregation in healthy males after consumption of a high-calorie diet.[29] The second study reported that ginger the co-administration of 1g of ginger with nifedipine resulted in an inhibition of platelet aggregation in normo- and hypertensive subjects.[28] However, in this study, when ginger was administered alone, there was no significant effect.

In contrast, two studies reported that 2-3.6g of ginger had no effect on measures of platelet aggregation in health adults.[26, 27] Moreover, Jiang et al.[26] found that the co-administration of 3.6g of ginger with 25mg of warfarin had no effect on the international normalized ratio (INR) or the pharmacokinetics and pharmacodynamics of warfarin in healthy male participants. Lumb et al.[27] also reported no significant effect on bleeding time, platelet count, and thromboelastography in a similar population. Bordia et al.[24] reported that 4g/day of ginger for three months did not affect platelet aggregation, fibrinogen, or fibrinolytic activity in patients with coronary artery disease; however, when participants were given a bolus dose of 10g ginger, there was a significant inhibition of platelet aggregation in patients with coronary artery disease.

The two studies that investigated the effect of ginger on thromboxane B_2 generation in healthy adults reported conflicting results. Srivastava et al.[19] reported that 5g of ginger over 7 days resulted in a 37% inhibition of thromboxane B_2 production ($p<0.01$), while Janssen et al.[25] found that 15g and 40g of raw and cooked ginger, respectively, had no effect when each were consumed for two weeks ($p=0.616$).

5.4.2 *Observational data*

Two observational studies investigated the association of ginger use and platelet-related adverse effects. Shalansky et al.[31] conducted a 16-week longitudinal study of 171 participants prescribed warfarin. During this period, participants were asked to record bleeding events as well as factors that the investigators hypothesised could influence INR and bleeding risk, including a selection of complementary

therapies. Of the 171 participants, 87 reported bleeding events with excessive bruising (41%) and nosebleeds (15%) being the two most commonly-reported events. The study reported a significant association between self-reported bleeding events and ginger (OR 6.63, 95% CI 3.49–12.61), as well as cayenne (OR 8.0, 95% CI 3.57–17.92), willow bark (OR 9.00, 95% CI 6.42–12.62), St. John's wort (OR 4.70, 95% CI 1.49–14.79), and coenzyme Q10 (OR 3.91, 95% CI 2.09–7.31). Upon further analysis, ginger (OR 3.20, 95% CI 2.42–4.24) and coenzyme Q10 (OR 3.69, 95% CI 1.88–7.24) were independently associated with self-reported bleeding events in a fully adjusted multivariate model. No complementary therapies were associated with a risk of abnormal INR.

In contrast, Leung et al.[32] surveyed 314 patients prescribed warfarin therapy, in which they retrospectively assessed self-reported bleeding events and exposure to factors that could influence bleeding risk and INR in the previous month. While only two patients reported using ginger during this period, the study authors determined that ginger, along with all other assessed complementary therapies, was not associated with bleeding risk or abnormal INR.

5.5 Discussion

Despite consistent *in vitro* data demonstrating that ginger compounds interact with several steps involved in platelet aggregation, the results of human studies are inconsistent. It is difficult to draw conclusions from these studies as a whole, due to the limited number of studies and their heterogeneous methods. These inconsistencies include the dose, dosing regimen, and formulation of ginger used, the timeframe studied, and the characteristics of subjects recruited (e.g. healthy vs. patients with chronic diseases).

Of the eight clinical trials analysed for this review, three found ginger affected measures of platelet aggregation[24, 28, 29] and one study found ginger reduced thromboxane B_2 production.[19] When the included studies were separated by patient medical background (e.g. healthy, hypertensive), no consistent treatment effect could be elucidated. However, there are several limitations that could limit the real-world applicability of these results.

First, Young et al.[28] reported that ginger had an effect only when it was combined with nifedipine, but not when it was ingested by itself. While not fully elucidated, it is thought that the anti-aggregation effect of nifedipine results from the inhibition of intracellular Ca2+, which attenuates platelet hyperactivity. [33] Other anti-platelet medications are not reported to possess this mechanism of action and therefore, these results might only be applicable to this combination.

Second, Verma et al.[29] found that ginger reduced a rise in platelet aggregation after a two week high-calorie diet when compared to control (high calorie diet plus placebo). However, it should be noted that this diet exceeded the participants' normal dietary intake (approximately 1600kcal increase in dietary intake, according to USDA food data[34]), which might make these results difficult to compare to patients who consume a eucaloric diet.

The third study reported a significant reduction in platelet aggregation when a bolus of 10g ginger was administered to patients with a confirmed myocardial infarction.[24] However, the same authors found a lower dose of 4g ginger had no effect in the same population when taken daily over three months.

A primary limitation of the studies reviewed is the lack of quantification or standardisation of bioactive compounds in the ginger preparations used. This could partly explain the inconsistent results. Previous research indicates that the concentration of the principal compounds within ginger, namely gingerol and shogaols, varies greatly depending on the storage and preparation of ginger products.[35, 36] This variation could result in significant differences in bioactive compounds between studies. For example, 6-shogaol is only present in appreciable amounts in dried or heated ginger as it is a degradation product of 6-gingerol.[37] Hence, preparations that used dried ginger are likely to have significantly different effects compared to raw ginger.

A final limitation relates to the clinical significance of ginger's potential anti-platelet effect. Several studies have reported that ginger is effective for nausea in multiple settings including morning sickness, motion sickness and chemotherapy-induced nausea and vomiting (CINV).[4, 5, 38] However, the majority of these studies used ginger doses that were considerably lower than those used in the studies included in this review. For example, in two recent reviews of the effect of ginger on morning sickness[5] and CINV[1], from a total of 19 studies, no study used a dose above 2g with most studies using a dosage around 1g. In contrast, the majority of studies in this review that found a significant effect on platelet aggregation used doses above 5g.[19, 24, 29] Young et al.[28] were the only exception in reporting 1g in combination with nifedipine to have an effect on platelet aggregation; however, when 1g of ginger was administered alone, there was no significant effect. Hence, further research in this area should investigate the effect of lower doses of ginger on platelet aggregation in order to determine if the potential effect of ginger on platelet aggregation is clinically

relevant when used as an adjuvant anti-nausea treatment during chemotherapy at doses shown to be effective in previous studies.

The two observational studies included in this review also reported conflicting results.[31, 32] This could be due to the differences in their study designs. One study undertook a retrospective analysis that could have resulted in recall bias,[32] while the other study undertook a prospective approach.[31] In the retrospective study,[32] only two patients from a cohort of 314 participants reported consuming ginger, both of whom reported experiencing bleeding events. Due to the limited sample of participants who consumed ginger, it is difficult to draw meaningful conclusions. Information regarding the dose of ginger consumed by participants was not reported in either observational study, which might further account for the difference in results.

While there was only one clinical trial investigating the interaction between ginger and warfarin, Jiang et al.[26] found no significant change to patient INR when ginger was administered for seven days. This is partially corroborated by the results of a study of Wistar rats in which a proprietary ginger formulation, in combination with warfarin, had no additive effect on whole blood clotting time, prothrombin time or activated partial thromboplastin time.[10] This is a particularly relevant finding, as ginger is routinely cited as potentially interacting with warfarin therapy.[39, 40] While further studies are required to investigate interaction of ginger and blood thinning medication, current evidence does not support an interaction.

The results of this review indicate that the role of ginger in platelet aggregation is unclear and therefore, future clinical trials are needed to further investigate this area, particularly in at-risk populations such as chemotherapy patients. However, until these

trials are undertaken, the effect of ginger on platelet aggregation cannot be confidently dismissed. Previous research has indicated that patient use of dietary supplements is often not reported to treating physicians. For example, a review of surveys that investigated the rate of non-disclosure of complementary and alternative medicines in chemotherapy patients found that between 40-50% of patients did not discuss these therapies with their physician.[41] Hence, where patients are at particular risk of bleeding, clinicians should ascertain patient consumption of dietary supplements and screen for any known potentiator of bleeding risk.

5.6 Conclusion

Due to the potential effects of ginger on platelet aggregation, ginger is a commonly-cited example of an herbal supplement that should be avoided in patients with thrombocytopenia, platelet function defects or coagulopathy, such as populations using ginger for its antiemetic effect in cancer chemotherapy. While *in vitro* data, as well as some clinical studies and epidemiological evidence suggest that ginger inhibits platelet aggregation, the evidence is equivocal with multiple limitations, particularly within the clinical data, which prevents firm recommendations being made. Limitations include the lack of standardisation of ginger preparations used, significant variations in dosage and time frame studied, and the high level of bias in the study designs used. Therefore, further research is needed to clearly define the safety, or otherwise, of ginger in patient population at increased risk of bleeding.

5.7 References

1. Marx WM, Teleni L, McCarthy AL, Vitetta L, McKavanagh D, Thomson D, et al. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. Nutrition Reviews. 2013;71(4):245-54. doi: 10.1111/nure.12016.
2. Chrubasik S, Pittler M, Roufogalis B. Zingiberis rhizoma: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine. 2005;12:684 - 701. PubMed PMID: doi:10.1016/j.phymed.2004.07.009.
3. Chaiyakunapruk N, Kitikannakorn N, Nathisuwan S, Leeprakobboon K, Leelasettagool C. The efficacy of ginger for the prevention of postoperative nausea and vomiting: a meta-analysis. Am J Obstet Gynecol. 2006;194(1):95-9. Epub 2006/01/04. doi: 10.1016/j.ajog.2005.06.046. PubMed PMID: 16389016.
4. Thomson M, Corbin R, Leung L. Effects of ginger for nausea and vomiting in early pregnancy: a meta-analysis. Journal of the American Board of Family Medicine : JABFM. 2014;27(1):115-22. Epub 2014/01/07. doi: 10.3122/jabfm.2014.01.130167. PubMed PMID: 24390893.
5. Lien HC, Sun WM, Chen YH, Kim H, Hasler W, Owyang C. Effects of ginger on motion sickness and gastric slow-wave dysrhythmias induced by circular vection. Am J Physiol Gastrointest Liver Physiol. 2003;284(3):G481-9. Epub 2003/02/11. doi: 10.1152/ajpgi.00164.2002. PubMed PMID: 12576305.

6. Lesho EP, Saullo L, Udvari-Nagy S. A 76-year-old woman with erratic anticoagulation. Cleve Clin J Med. 2004;71(8):651-6. Epub 2004/09/29. PubMed PMID: 15449760.

7. Dorso CR, Levin RI, Eldor A, Jaffe EA, Weksler BB. Chinese food and platelets. N Engl J Med. 1980;303(13):756-7. Epub 1980/09/25. PubMed PMID: 7402279.

8. Srivastava KC. Aqueous extracts of onion, garlic and ginger inhibit platelet aggregation and alter arachidonic acid metabolism. Biomed Biochim Acta. 1984;43(8-9):S335-46. Epub 1984/01/01. PubMed PMID: 6440548.

9. Srivastava KC. Isolation and effects of some ginger components of platelet aggregation and eicosanoid biosynthesis. Prostaglandins Leukot Med. 1986;25(2-3):187-98. Epub 1986/12/01. PubMed PMID: 3103137.

10. Weidner MS, Sigwart K. The safety of a ginger extract in the rat. Journal of Ethnopharmacology. 2000;73(3):513-20.

11. Elting LS, Rubenstein EB, Martin CG, Kurtin D, Rodriguez S, Laiho E, et al. Incidence, Cost, and Outcomes of Bleeding and Chemotherapy Dose Modification Among Solid Tumor Patients With Chemotherapy-Induced Thrombocytopenia. Journal of Clinical Oncology. 2001;19(4):1137-46.

12. Liao YR, Leu YL, Chan YY, Kuo PC, Wu TS. Anti-platelet aggregation and vasorelaxing effects of the constituents of the rhizomes of Zingiber officinale. Molecules. 2012;17(8):8928-37. Epub 2012/07/28. doi: 10.3390/molecules17088928. PubMed PMID: 22836212.

13. Koo KL, Ammit AJ, Tran VH, Duke CC, Roufogalis BD. Gingerols and related analogues inhibit arachidonic acid-induced human platelet serotonin

release and aggregation. Thromb Res. 2001;103(5):387-97. Epub 2001/09/13. PubMed PMID: 11553371.

14. Flynn DL, Rafferty MF, Boctor AM. Inhibition of human neutrophil 5-lipoxygenase activity by gingerdione, shogaol, capsaicin and related pungent compounds. Prostaglandins Leukot Med. 1986;24(2-3):195-8. Epub 1986/10/01. PubMed PMID: 3467378.

15. Thomson M, Al-Qattan KK, Al-Sawan SM, Alnaqeeb MA, Khan I, Ali M. The use of ginger (Zingiber officinale Rosc.) as a potential anti-inflammatory and antithrombotic agent. Prostaglandins Leukot Essent Fatty Acids. 2002;67(6):475-8. Epub 2002/12/07. PubMed PMID: 12468270.

16. Tjendraputra E, Tran VH, Liu-Brennan D, Roufogalis BD, Duke CC. Effect of ginger constituents and synthetic analogues on cyclooxygenase-2 enzyme in intact cells. Bioorganic chemistry. 2001;29(3):156-63. Epub 2001/07/05. doi: 10.1006/bioo.2001.1208. PubMed PMID: 11437391.

17. Nie H, Meng LZ, Zhang H, Zhang JY, Yin Z, Huang XS. Analysis of anti-platelet aggregation components of Rhizoma Zingiberis using chicken thrombocyte extract and high performance liquid chromatography. Chin Med J (Engl). 2008;121(13):1226-9. Epub 2008/08/20. PubMed PMID: 18710644.

18. Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD, Tran VH, Duke CC. Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. Thrombosis Research. 2003;111(4–5):259-65. doi: 10.1016/j.thromres.2003.09.009.

19. Srivastava KC. Effect of onion and ginger consumption on platelet thromboxane production in humans. Prostaglandins Leukot Essent Fatty Acids. 1989;35(3):183-5. Epub 1989/03/01. PubMed PMID: 2710801.

20. Nievergelt A, Marazzi J, Schoop R, Altmann KH, Gertsch J. Ginger phenylpropanoids inhibit IL-1beta and prostanoid secretion and disrupt arachidonate-phospholipid remodeling by targeting phospholipases A2. J Immunol. 2011;187(8):4140-50. Epub 2011/09/13. doi: 10.4049/jimmunol.1100880. PubMed PMID: 21908733.

21. Moher D, Liberati A, Tetzlaff J, Altman DG, The PG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009;6(7):e1000097. doi: 10.1371/journal.pmed.1000097.

22. National Health and Medical Research Council. NHMRC additional levels of evidence and grades for recommendations for developers of guidelines. Commonwealth of Australia: National Health and Medical Research Council 2009.

23. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions The Cochrane Collaboration; 2011. Available from: www.cochrane-handbook.org.

24. Bordia A, Verma SK, Srivastava KC. Effect of ginger (Zingiber officinale Rosc.) and fenugreek (Trigonella foenumgraecum L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. Prostaglandins Leukot Essent Fatty Acids. 1997;56(5):379-84. Epub 1997/05/01. PubMed PMID: 9175175.

25. Janssen PL, Meyboom S, van Staveren WA, de Vegt F, Katan MB. Consumption of ginger (Zingiber officinale roscoe) does not affect ex vivo platelet thromboxane production in humans. Eur J Clin Nutr. 1996;50(11):772-4. Epub 1996/11/01. PubMed PMID: 8933126.

26. Jiang X, Williams KM, Liauw WS, Ammit AJ, Roufogalis BD, Duke CC, et al. Effect of ginkgo and ginger on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects. Br J Clin Pharmacol. 2005;59(4):425-32. Epub 2005/04/02. doi: 10.1111/j.1365-2125.2005.02322.x. PubMed PMID: 15801937; PubMed Central PMCID: PMC1884814.

27. Lumb AB. Effect of dried ginger on human platelet function. Thromb Haemost. 1994;71(1):110-1. Epub 1994/01/01. PubMed PMID: 8165628.

28. Young HY, Liao JC, Chang YS, Luo YL, Lu MC, Peng WH. Synergistic effect of ginger and nifedipine on human platelet aggregation: a study in hypertensive patients and normal volunteers. Am J Chin Med. 2006;34(4):545-51. Epub 2006/08/03. doi: 10.1142/s0192415x06004089. PubMed PMID: 16883626.

29. Verma SK, Singh J, Khamesra R, Bordia A. Effect of ginger on platelet aggregation in man. Indian J Med Res. 1993;98:240-2. Epub 1993/10/01. PubMed PMID: 8119760.

30. Janssen PLTMK, Meyboom S, Van Staveren WA, De Vegt F, Katan MB. Consumption of ginger (Zingiber Officinale Roscoe) does not affect ex vivo platelet thromboxane production in humans. European Journal of Clinical Nutrition. 1996;50(11):772-4.

31. Shalansky S, Lynd L, Richardson K, Ingaszewski A, Kerr C. Risk of warfarin-related bleeding events and supratherapeutic international normalized ratios associated with complementary and alternative medicine: a longitudinal analysis. Pharmacotherapy. 2007;27(9):1237-47. Epub 2007/08/29. doi: 10.1592/phco.27.9.1237. PubMed PMID: 17723077.

32. Leung VW, Shalansky SJ, Lo MK, Jadusingh EA. Prevalence of use and the risk of adverse effects associated with complementary and alternative medicine in a cohort of patients receiving warfarin. Ann Pharmacother. 2009;43(5):875-81. Epub 2009/04/30. doi: 10.1345/aph.1L631. PubMed PMID: 19401475.

33. Shih CY, Lin MH, Fan HC, Chen FC, Chou TC. Mechanisms of antiplatelet activity of nifedipine: role of peroxisome proliferator-activated receptor-beta-gamma-dependent processes. Journal of hypertension. 2014;32(1):181-92. Epub 2013/10/16. doi: 10.1097/hjh.0000000000000007. PubMed PMID: 24126710.

34. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 27. 2014.

35. Schwertner H, Rios D, Pascoe J. Variation in concentration and labeling of ginger root dietary supplements. Obstet Gynecol. 2006;107:1337 - 43. PubMed PMID: doi:10.1097/01.AOG.0000217697.33787.8c.

36. Schwertner HA, Rios DC. High-performance liquid chromatographic analysis of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in ginger-containing dietary supplements, spices, teas, and beverages. J Chromatogr B Analyt

Technol Biomed Life Sci. 2007;856(1-2):41-7. Epub 2007/06/15. doi: 10.1016/j.jchromb.2007.05.011. PubMed PMID: 17561453.

37. Srinivasan R, Wong WH, Pichika MR. Impact of extraction processes on the 6-shogaol content in Zingiber officinale (Halia Penang) and its antiproliferative activities on human colorectal cancer cell lines (HT-29). Planta Med. 2011;77(05):112. doi: 10.1055/s-0031-1273641.

38. Ryan JL, Heckler CE, Roscoe JA, Dakhil SR, Kirshner J, Flynn PJ, et al. Ginger (Zingiber officinale) reduces acute chemotherapy-induced nausea: a URCC CCOP study of 576 patients. Support Care Cancer. 2012;20(7):1479-89. Epub 2011/08/06. doi: 10.1007/s00520-011-1236-3. PubMed PMID: 21818642; PubMed Central PMCID: PMCPMC3361530.

39. Heck AM, DeWitt BA, Lukes AL. Potential interactions between alternative therapies and warfarin. American Journal of Health-System Pharmacy. 2000;57(13):1221-30.

40. Miller LG. Herbal medicinals: selected clinical considerations focusing on known or potential drug-herb interactions. Archives of internal medicine. 1998;158(20):2200-11. Epub 1998/11/18. PubMed PMID: 9818800.

41. Davis EL, Oh B, Butow PN, Mullan BA, Clarke S. Cancer patient disclosure and patient-doctor communication of complementary and alternative medicine use: a systematic review. Oncologist. 2012;17(11):1475-81. Epub 2012/08/31. doi: 10.1634/theoncologist.2012-0223. PubMed PMID: 22933591; PubMed Central PMCID: PMC3500370

Part Two: Research studies and results

The results of the systematic literature reviews included in Part One of this thesis identified multiple areas where further research is required. In Part Two of this thesis, the aim was to address these gaps in the literature by conducting four research studies. To summarise the nature of these four studies, using an *in silico* model of the murine 5-HT_3 receptor, the binding characteristics of the principle compounds within ginger were explored in Chapter 7. Chapter 8 describes the results of an HPLC analysis that quantified the concentration of bioactive compounds within a variety of commercial ginger products. The effect of a standardized ginger extract was investigated in Chapter 8 and 9 by conducting a double blind, randomized, placebo-controlled trial. Finally, in Chapter 10, current barriers, needs and behaviours relating to the implementation of potentially therapeutic dietary supplements such as ginger were elicited from a survey of 370 healthcare professionals. The referencing style and manuscript structure of each chapter are in accordance with the respective journal guidelines.

Chapter 6. In silico investigation into the interaction between murine 5-HT$_3$ receptor and principle compounds of ginger (Zingiber officinale).

X-ray crystallography produces a three-dimensional map of the atomic coordinates of biomolecules with or without their associated ligands, providing a basis for further computation analysis of biomolecular interactions. For example, molecular docking is a computer-based tool commonly used in drug design whereby the potential binding affinity of potential drug candidates to a biological target can be investigated. 5-HT$_3$ antagonism is thought to be one of the primary mechanisms by which the active compounds (e.g gingerols and shogaols) within ginger potentially reduce CINV. However, it is currently unclear how these active compounds interact with this receptor, with some evidence suggesting these compounds bind to a currently unidentified site that differs to serotonin and other known 5-HT$_3$ antagonists (e.g. ondansetron). Recently, the crystal structure of the murine 5-HT$_3$ receptor was solved, allowing for an *in silico* investigation of the binding characteristics of the primary ginger compounds within the newly-solved 5-HT$_3$ receptor. As discussed in detail in this chapter, the binding affinities of several bioactive ginger compounds, along with an array of relevant compounds of interest, were investigated in two potential binding sites. The results of this study provide information regarding favourable binding sites, potential binding conformations and key residues required for binding. These are discussed in relation to previously cited residues identified as potential important for binding.

Citation: Marx WM, Isenring E, Lohning A. In silico investigation into the interaction between murine 5-HT3 receptor and principle compounds of ginger (Zingiber officinale). European Journal of Medicinal Chemistry. Impact factor: 3.447; Intended submission: December, 2015

Relevant Appendices:

- Appendices A. GRID Analysis and Structural Similarity Map,
- Appendices B. Fasta Sequencing of Murine and Human 5-HT3 Receptor

6.1 Abstract

Gingerols and shogaols are the primary compounds within ginger (*Zingiber officinale*) and have been demonstrated *in vitro* to exert 5-HT_3 antagonism which could benefit chemotherapy-induced nausea and vomiting. However, the site and mechanism of action on the receptor by which these compounds bind to the 5HT3 receptor has not been fully elucidated although research indicates they may bind to a currently unidentified allosteric binding site. Using *in silico* analyses of the recently available murine 5-HT_3 receptor, we conducted a GRID analysis ofsite environment and characteristics of these compounds along with other positive and negative controls within the serotonin binding site. Docking results were compared to those using a proposedallosteric binding site situated at the interface between the transmembrane region and the extracellular domain. Our results correlated well with previous site-directed mutation studies in identifying key binding site residues. We have identified residues which may be important for binding the ginger compounds. Overall, our results suggest that the ginger compounds and their structural analogues possess a high binding affinity to both sites. . While these compounds contain structural moieties which likely contribute to their high docking scores *in silico* and, notwithstanding the limitations of theoretical analyses, it is also conceivable that these compounds could act both competitively or non-competitively as has been shown for other modulators of CYS loop receptors.

6.2 Introduction

One of the primary pathways in the pathology of chemotherapy-induced nausea and vomiting is the stimulation of vagal afferent nerves via the 5-HT_3 receptors due to abnormally high levels of serotonin released from the mucosal enterochromaffin cells of the gut.[1] Serotonin acts allosterically to activate the receptor since its binding site is distinct from the transmembrane region where channel opening occurs. A number of agonists and antagonists have been identified which are able to displace serotonin.[2] The "setron" class of anti-emetic medications, including ondansetron as well as the more recently introduced polanosetron and granisetron, have significantly improved control of chemotherapy-induced nausea and vomiting due to their action as a competitive antagonist of the 5-HT_3 receptor.[1] Additionally, as established for other CYS loop receptors, allosteric modulators have been observed which exert an effect on the activity of the receptor while in the presence of serotonin (or other compounds) bound at the orthosteric binding site.[3,4] For example, reports of a putative 'alcohol' binding site followed observations of enhancement of agonist displacement of ^{3}H-granisetron in the presence of trichloroethanol at 5-HT_3 receptor conductance by alcohols following displacement by other agonists. These effects were however species dependent.[4]

Empirical evidence from *in vitro* and clinical data suggest that ginger may be an effective treatment against several types of nausea including morning sickness, motion sickness and chemotherapy-induced nausea and vomiting.[5,6] Gingerols are the principle class of compounds within non-volatile component of ginger. Thermal treatment during dehydration or other manufacturing processes convert some gingerols to the more

oxidised shogaols or dehydroshogaols.[7] Both shogaols and gingerols contain a second double bond on the opposite site of the carbonyl. In contrast to the gingerols, both the shogaols and dehydroshogaols are α,β-unsaturated carbonyl compound.[8]

Preclinical studies have also begun to elucidate the mechanisms by which these compounds may exert a potentially beneficial effect on nausea and have found multiple viable pathways.[9-11] Previous studies have demonstrated that the principle compounds in ginger, gingerols and shogaols, are able to inhibit 5-HT_3 mediated signaling and that this interaction could be mediated through a currently unidentified binding site.[9,10] Abdel-Aziz et al.[9] conducted a series of *in vitro* studies which found that [6]-shogaol, [8]-gingerol, [10]-gingerol, and [6]-gingerol were able to inhibit 5-HT_3-induced contractions of the isolated guinea-pig ileum and that this was likely via a distinct binding site due to the same compounds inability to displace the competitive antagonist, [^{3}H]GR65630 from its binding site. These findings were corroborated by an *in vitro* study by Walstab et al.[10] that investigated the effect of three ginger extracts as well as [6]-gingerol and [6]-shogaol on the activation of human 5-HT_3 receptors. The results indicated that ginger was able to inhibit the activation of 5-HT_3 receptors and this was likely via non-competitive mechanisms. As Walstab et al.[10] noted, when combined with standard 5-HT_3 antagonists, the non-competitive binding of ginger could potentially provide an additive effect to the control of nausea and vomiting in clinical practice.

The 5-HT_3 receptor is a pentameric, neurotransmitter-gated ion channel of the CYS loop family. Five distinct subunits (A to E) have been identified to date whereby A, B, C & E are similar while subunit 5-HT_{3D} lacks an amino terminal CYS loop. The arrangement of subunits in the functioning unit has five-fold symmetry of subunits, either arranged homo or heteromerically around a cation-specific, water filled central pore. Only the A subunit has been shown to form functional homomeric receptors and, importantly, the presence of the A subunit was required in all receptors. It is noteworthy that heteromeric receptors contain more possible sites for allosteric modulation than homomeric receptors.[12]

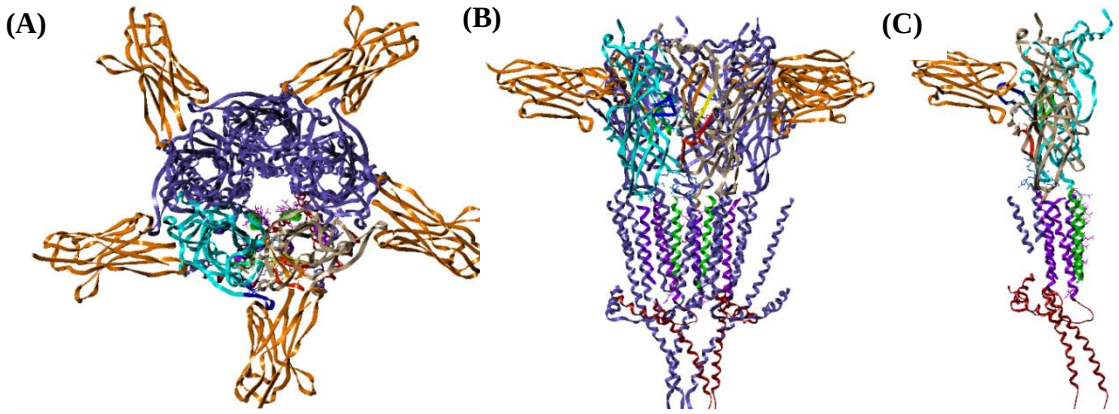


Figure 6-1 Homopentameric murine 5-HT3 receptor with VHH nanobodies. Top view (A) and side view (B) of homopentameric murine 5-HT3 receptor with VHH nanobodies (orange ribbons) (pdb entry 4pir). The backbone of 5-HT3 is depicted as a purple ribbon except for the principle subunit extracellular domain (ECD) in cyan ribbon and the complementary subunit ECD in beige ribbon. Pore-lining M2 helices in the transmembrane domain of principle and complementary subunits in green ribbon (C); Two of the 5 subunits extracted for analysis representing principle and complementary subunits (A+A-).

Recently, the crystal structure of the murine 5-HT_3 receptor in the apo (or unbound) form was solved using X-ray crystallography at 3.5Å resolution.[13] VHH nanobodies, derived from single subunit llama immunoglobulins, were used to help

stabilize the receptor during crystallization. In the crystal structure, these are shown to bind radially at the intersubunit interface (Figure 6-1in orange). Hassaine et al.[13] found that the binding of the VHH nanobodies resulted in inhibition, possibly stabilizing a non-conducting conformation. Despite the relatively low resolution, knowledge of the three dimensional structure provides the necessary information to conduct an *in silico* study with the aim of investigating the binding characteristics of the primary compounds within ginger on the 5-HT_3 receptor. In particular, this study compared the binding interactions of the active ginger compounds at the serotonin site with those at the proposed allosteric binding site. This will provide additional insight into the nature of the binding characteristics of gingerol and shogaol compounds in relationship to the 5-HT_3 receptor.

6.3 Results and discussion

The binding interactions of each compound were investigated using the recently solved crystal structure of the murine 5-HT_3 receptor (PDB entry 4pir). Although the crystal structure depicts a homomeric 5-HT_{3A} pentamer, two subunits (A+A-) were extracted for analysis since both the serotonin and allosteric binding sites are located at or near this interface.

As shown in Figure 6-2, the serotonin binding site is at the interface between two adjacent subunits.[2,13] The proposed allosteric binding site is situated at the interface between the transmembrane region and the extracellular domain.[14] The location of the latter site was delineated from site-directed mutagenesis studies[14]

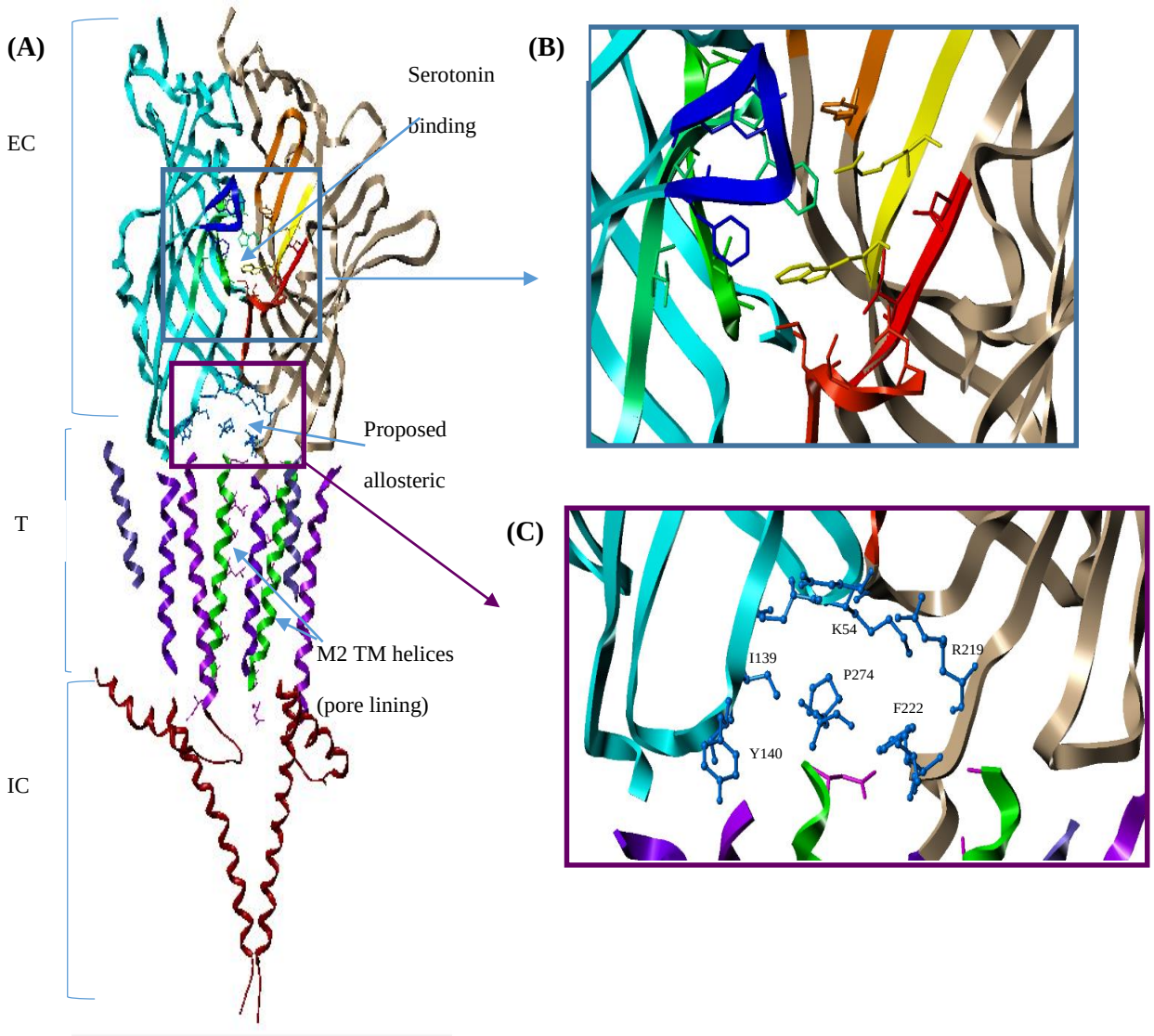
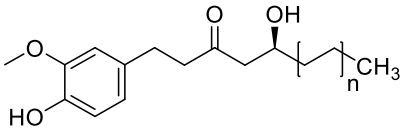


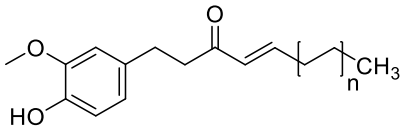
Figure 6-2 (A) Principle (cyan ribbon) and complementary (beige ribbon) subunits of 5HT-3_A receptor (A+A-). ECD (extracellular domain containing orthosteric binding site and allosteric site at interface with TMD; TMD (transmembrane domain containing M1-M4 TM helices with M2 in green containing pore-facing residues ion magenta); ICD Intracellular domain. (B) Orthosteric (serotonin) binding site: Principle subunit (cyan ribbon): Loop A (N97,N101 green); Loop B (T52,54, W156 green-blue); Loop C (F199, Y207 blue). Complementary subunit (beige ribbon): Loop D (W63, R65, Y68 yellow); Loop E (Y124 orange); Loop F (D177, S179, V180 red-orange); Loop G (D42, 44 red). (C) Proposed allosteric site: Principle subunit (cyan ribbon) Complementary subunit (beige ribbon). Key residues thought to be important for binding non-competitive antagonists (blue ball & stick).

A ligand database of 25 compounds was prepared comprising 6,8 and 10-gingeraol and 6 and 10-shogaol, serotonin as well as a number positive and negative controls (Figure 6-3).

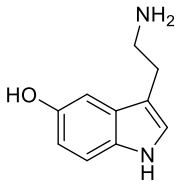
Figure 6-3 Structural diagrams of included ligands



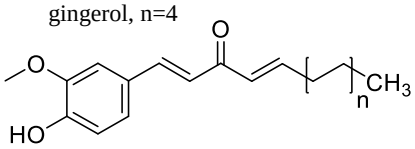
6-gingerol, n=2; 8-gingerol, n=3; 10-gingerol, n=4



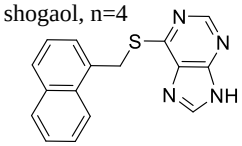
6-shogaol, n=2; 8-shogaol, n=3; 10-shogaol, n=4



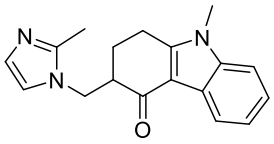
serotonin



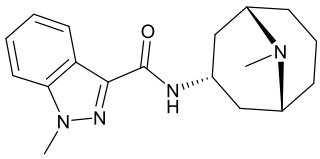
6-dehydroshogaol, n=2; 8-dehydros-hogaol, n=3; 10-dehydroshogaol, n=4



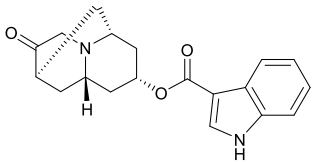
(6-[(1-naphthylmethyl)thio] -9H-purine) (PU02)



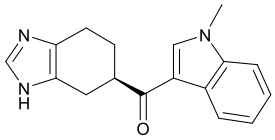
ondansetron



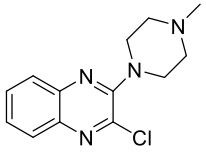
granisetron



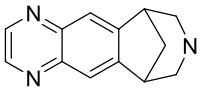
dolasetron



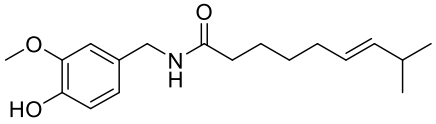
romasetron



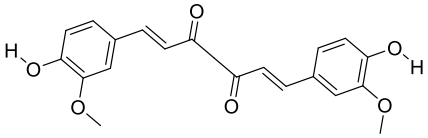
VUF10166



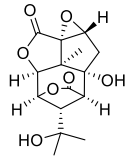
varenicline



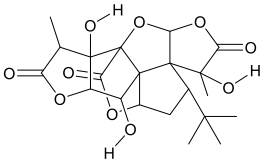
capsaicin



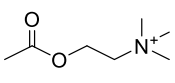
curcumin



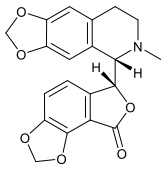
picrotoxin



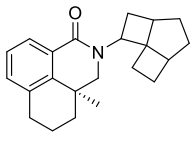
gingkolide



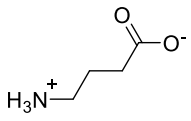
acetylcholine



bicuculline



palonosetron



γ-hydroxybutyruic acid (GABA)

Positive controls included known competitive antagonists such as ondansetron known to bind at the serotonin binding site as well as known non-competitive antagonists, such as picrotoxin, which are likely to bind at an allosteric or alternate site. In addition, we included structural analogs of the gingerols (capsaicin and curcumin) and decoy molecules such as bicuculline not known to bind to the 5-HT_3 receptor.

A GRID analysis was performed to identify sites of strong binding interaction between the receptor and a range of small probes simulating various functional groups of the ligands (*see appendix for GRID data*). Ligands were subsequently docked into the two sites to compare binding interactions at each site.

The docking results are presented in Table 6-1. Surflex-Dock ranks ligands in order of highest binding interaction to lowest by applying scoring functions, taking into account non-bonded interactions between the ligand and target, including hydrophobic, polar, electrostatic, van der Waal and entropic considerations. Consensus scoring (C_{score}) calculates scores across all 4 scoring functions. C_{scores} are between 0-5, with a C_{score} of 5 reflecting complete consensus of the pose binding score across all scoring functions while a lower score indicating less consensus. The total score is expressed as $-\log K_D$ to represent binding affinity. The lower the dissociation constant, K_D, the stronger the binding. When expressed as $-\log K_D$ a higher positive value reflects stronger binding.

Total scores comprise the sum of a positive 'polar' contribution and a negative 'crash' score. The 'crash' factor denotes the degree of inappropriate penetration of ligand atoms within the binding site. While the 'polar' contribution incorporates the hydrogen

bonding and other non-bonded interaction terms. Those ligands able to interact strongly to target residue atoms are likely to have higher total scores unless negative steric factors predominate. The degree of hydrogen bonding is included in Table 6-1 as one contributing factor towards overall total score.

Table 6-1 Surflex-Dock results for Serotonin and Allosteric Sites

	Serotonin Site				**Allosteric Site**			
Name	**Total score**	**Cscore**	**HB[a]**	**Key residues[b]**	**Total score**	**Cscore**	**HB[a]**	**Key residues[b]**
Ginger Ligands								
6-gingerol	8.7	1	3	E209 **R65**	8.26	1	4	R219 Q56 F222 E53
8-gingerol	10.25	5	4	**T154** E209 **R65**	8.84	5	3	E53 R219 F222
10-gingerol	10.81	4	5	**T154** E209 K211 **T152**	8.26	1	5	T280 I139 E53 Q56
6-shogaol	8.38	0	2	**N101** **W156**	6.52	0	3	E53 F222 Q56
8-shogaol	9.06	5	4	**R65** **S155** **T154**	7.19	2	2	K54 F222
10-shogaol	9.34	2	2	**T152** **N101**	8.29	5	1	F222

6-dehdroshogaol	6.97	0	3	**T152** K211 **N101**	6.28	0	3	E53 Q56 K54 E186
8-dehdroshogaol	8.56	0	3	**L157** **Y207** **N101**	6.61	0	1	
10-dehdroshogaol	9.07	2	2	**L157** **N101**	6.85	4	3	E53 Q56 K54
Native Ligand								
Serotonin (5-hydroxytryptamine)	5.63	0	5	E173 S176 **D42** **D177**	6.02	0	4	Q184 E53 D138 L137
Competitive Antagonists								
Ondansetron	5.22	0	1	**T154**	4.85	0	1	Q56
Granisetron	5.51	0	1	E209	4.87	0	0	
Dolasetron	6.9	0	3	**R65** **T154**	5.43	1	0	
Ramosetron	6.48	2	1	**T154**	5.65	2	2	P274 Q56
Palonosetron	5.74	0	1	**R65**	5.10	0	0	
VUF10166	5.13	1	1	**R65**	5.80	4	0	
Varenicline (from 5AIN.pdb)	5.09	3	2	**R65** **N101**	4.23	3	1	P274
PU02	5.8	5	3	**D177** **S179**	4.33	2	1	D138

Structural Analogues of ginger actives								
Capsaicin	8.54	4	4	**R65** **N101**	9.23	1	3	K54 R219 F222
Curcumin	8.77	1	9	**R65** **T154** S155 **D177** **S179**	7.02	0	3	R219 E53 E186
Non-Competitive Ligands								
Picrotoxin	4.77	1	4	E102 S150 S136 N148	4.96	0	4	Y46 N183 S136
Ginkgolide	4.25	5	7	K211 S150 E102 **T152** **N101**	3.94	3	3	T280 D138 I139
Decoys								
Acetylcholine	4.9	1	0		4.95	3	1	Q56
Bicuculline	7.09	2	1	**R65**	6.01	1	3	F222 T280 Q56
GABA	4.9	1	3	**W156** **R65**	4.76	1	3	Q56 K54 E53

[a] number of hydrogen bonds; [b] target residues hydrogen bonding to ligand; Blue colour indicates residues previously identified as important for binding.[13]

Overall the c_{scores} for the ginger compounds were consistently higher at the allosteric site while the c_{score} was more variable at the serotonin site for the ginger compounds overall. This trend was repeated with the structural analogs inferring that this

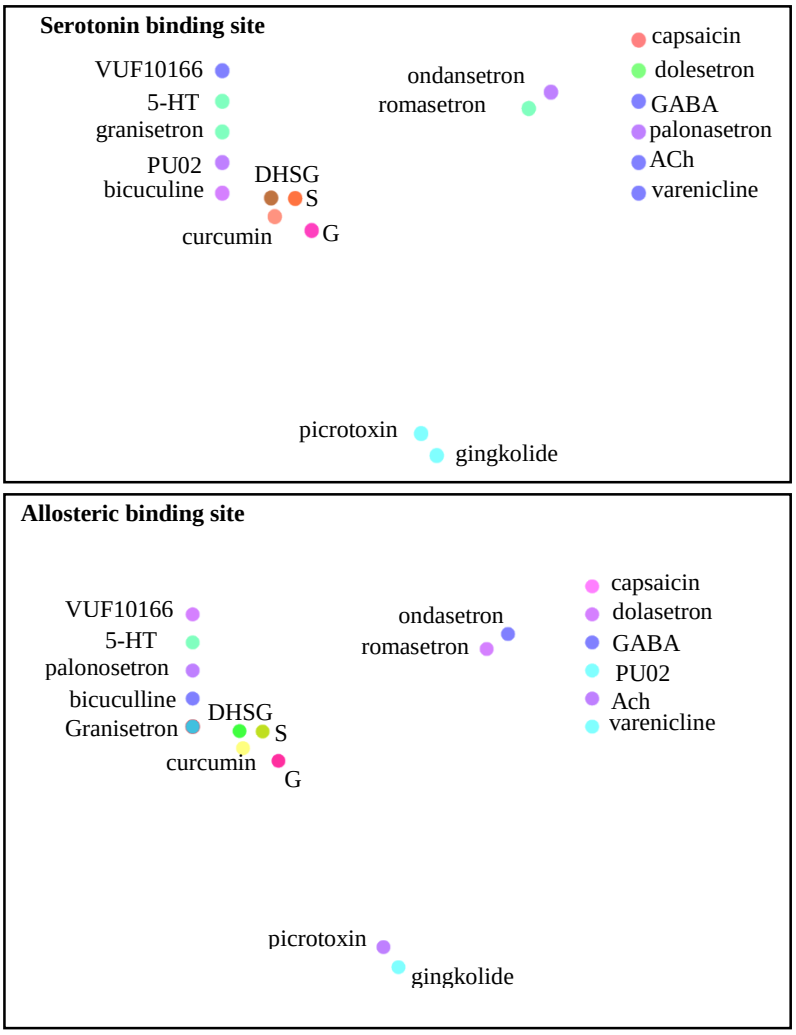


Figure 6-4 Structural similarity maps at each binding site coloured by total score. Cyan, blue and purple indicate low total scores. Green and yellow indicate moderate scores while magenta/red ere high scoring. G = gingerols, S = shogaols, DHSG = hehydroshogaols; Ach = acetylcholine; 5-HT = serotonin..

structural family of compounds binds more favorably at the allosteric site. In contrast, there was a higher consensus for serotonin and the setron antagonists at the serotonin

binding site. Consensus was high at both sites for the decoys, consistently performing poorly in their total scores.

Figure 6-4 is a structural similarity plot depicting the relatedness of molecules in the database and coloured according to total scores in each site. The plots represent a principle component analysis based on UNITY molecular fingerprints. Each molecule is characterized by a set of fragments (represented in binary). Points near one another represent similar compounds (a similar UNITY fingerprint). Points far from one another represent dissimilar compounds. Points around the edge of the map represent compounds that are not like any other compounds used to generate the map. The colours range from magenta/red as high scoring through to blue and cyan lowest scoring (*see appendix for colour key*). Structurally, the two non-competitive antagonists (NCA), gingkolide and picrotoxin are clearly most distinctly unrelated to the other ligands though somewhat similar to each other. These two scored the lowest in the serotonin site which is consistent with their action as NCAs. The ginger compounds are clustered with curcumin although capsaicin, another structural analog, is clustered elsewhere. This is most likely due to presence of the amine functional group. Serotonin is clustered with the amine-containing GABA, capsaicin, acetylcholine as well as one of the setrons, dolasetron. Of all the setrons, dolasetron is the only one with an indole moiety similar to serotonin which may explain the positioning of this ligand. The remaining larger ring system ligands were clustered together with bicuculline and VUF1016. A cluster analysis of 20 poses for each ligand at both sites showed that where there was one preferred orientation and that the highest scoring pose from each cluster was found within the preferred orientation.

6.3.1 *Serotonin binding site*

It is established that serotonin site has a high degree of hydrophobic character and our results are in agreement with this description.[13] Figure 6-5-A depicts the GRID results predicting strong sites of interaction with a hydrophobic probe. A contour level of -1.5kcal/mol is indicative of a lipophilic region. Serotonin is observed to dock into a more polar region than the setron compounds which is as expected given their greater degree of lipophilicity. A strong site of interaction correlates well with the position of the aromatic ring and alkyl chain of docked [6]-gingerol (Figure 6-5-B).

The orientation of all docked ligands are presented in Figure 6-6-A. Serotonin bound to a distinctly polar location closer to complementary subunit and shared common binding interactions only with curcumin and dolasetron (Figure 6-6-B).

The majority of ligands, including the ginger compounds, occupied a site more interior and hydrophobic than that bound by serotonin (Figure 6-6-B and C). Despite the 3 apparent hydrogen bonds with D177 and one each between S179 and W165, the total score for serotonin was lower than all ginger compounds, structural analogs, granisetron, dolasetron and romasetron.

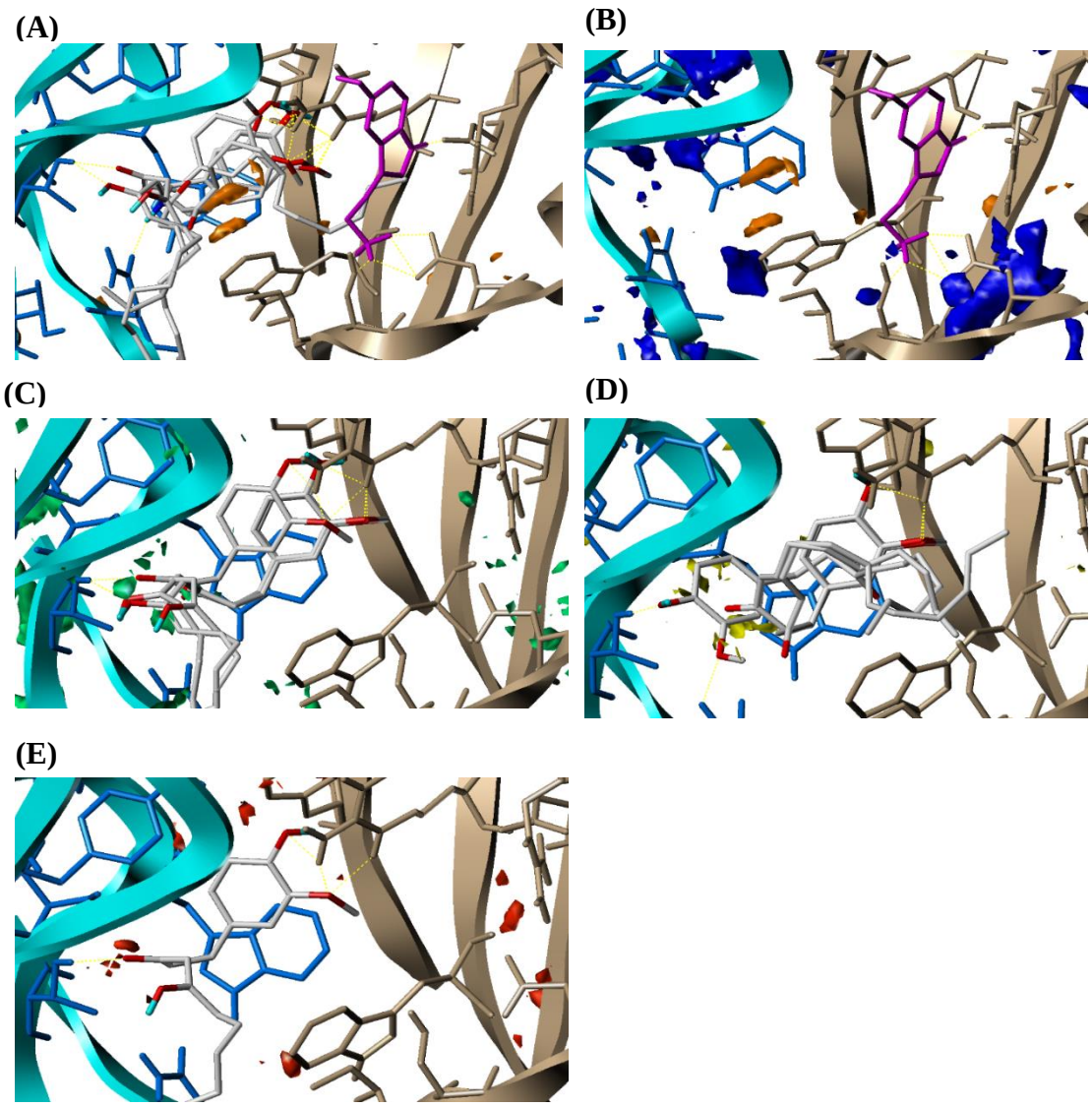


Figure 6-5. Serotonin binding site with (A) Docked ginger compounds (atom types) and 5HT (magenta). Hydrophobic probe contoured at -1.5kcal/mol. (B) Serotonin docked with amine probe contoured at -15kcal/mol (C) Docked poses of 6,8 and 10G with alkyl hydroxyl probe contours (-10.5kcal/mol,greenblue). (D) Docked poses of 6,8 and 10S with alkyl hydroxyl probe contours (-10.5 kcal/mol, yellow) (E) Docked poses of 10G with carbonyl oxygen probe contours (-7 kcal/mol, redorange)

The serotonin site is lined with a number of aromatic residues (an aromatic box). A GRID analysis showed the three particularly strong sites of interaction with a hydrophobic probe. (Figure 6-5-A) and correlate well with the docked positions of hydrophobic moieties of the ligands such as the alkyl tails of the ginger compounds and the aromatic ring systems of other compounds. A GRID analysis using an amine cationic probe showed excellent correlation to the site of the docked ammonium group of serotonin (Figure 6-5-B).

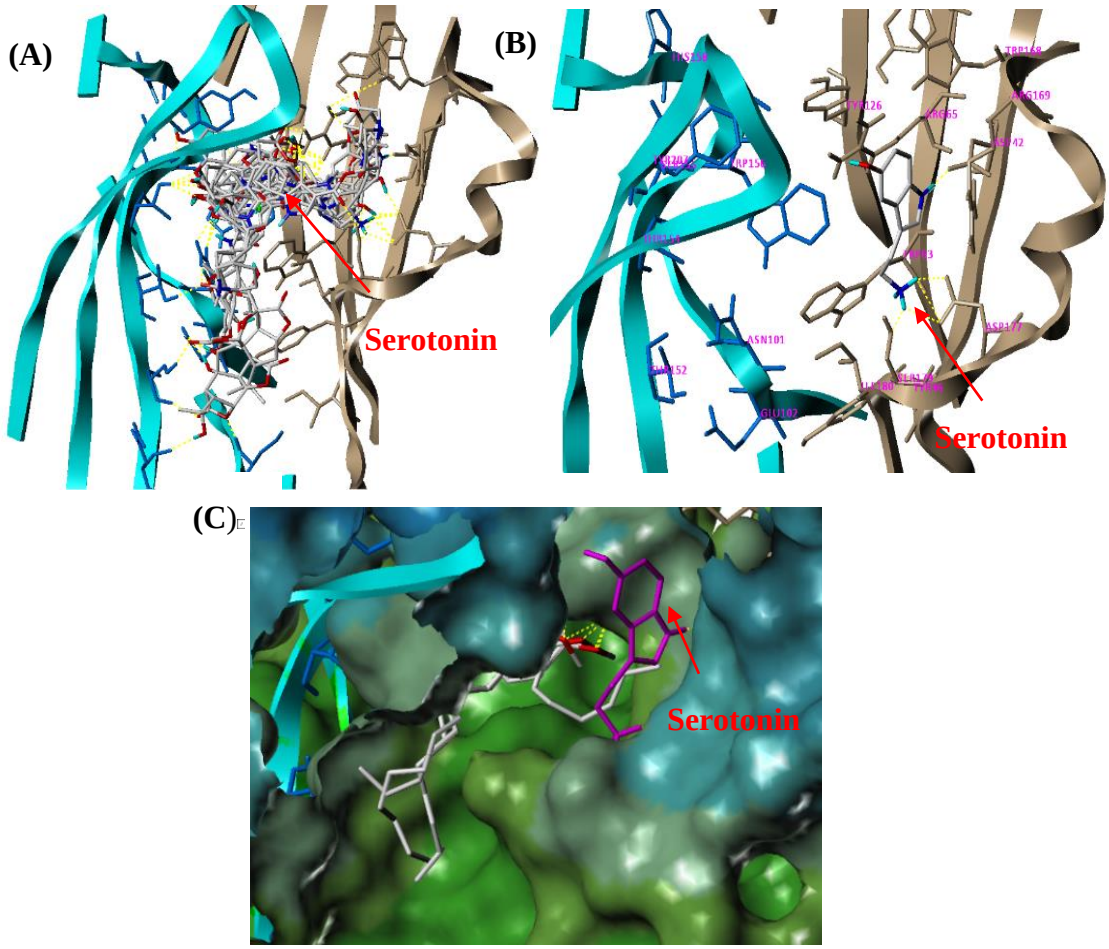


Figure 6-6. Serotonin binding site with key residues labelled. (A) Superimposition of highest scoring poses of all gingerols compounds (B) Serotonin (atom colours) docked within the site showing hydrogen bonds (yellow dashes). S(C) Serotonin (magenta) docked in a polar site closer to complementary subunit (blue surface) compared to the more interior, hydrophobic site (green surface) occupied by most other ligands.

The gingerols compounds are distinguished from the other more oxidized ginger compounds by the presence of an alkyl hydroxyl group. Sites of polar interactions with an alkyl hydroxyl group; this correlate well with the position of alkyl hydroxyl probe of [6]-gingerol. Predicted sites of binding interactions of a phenyl hydroxyl probe is less well correlated with the position of the same group in [6]-gingerol. (Figure 6-5-B)

A GRID analysis with an alkyl hydroxyl probe shows a site of strong interaction within this site at the position of the docked gingerol's hydroxyl group (Figure 6-5-C). Similarly the docked positions of an aryl hydroxyl and carbonyl oxygen correlated well with the sites predicted by GRID (Figure 6-5-D and E).

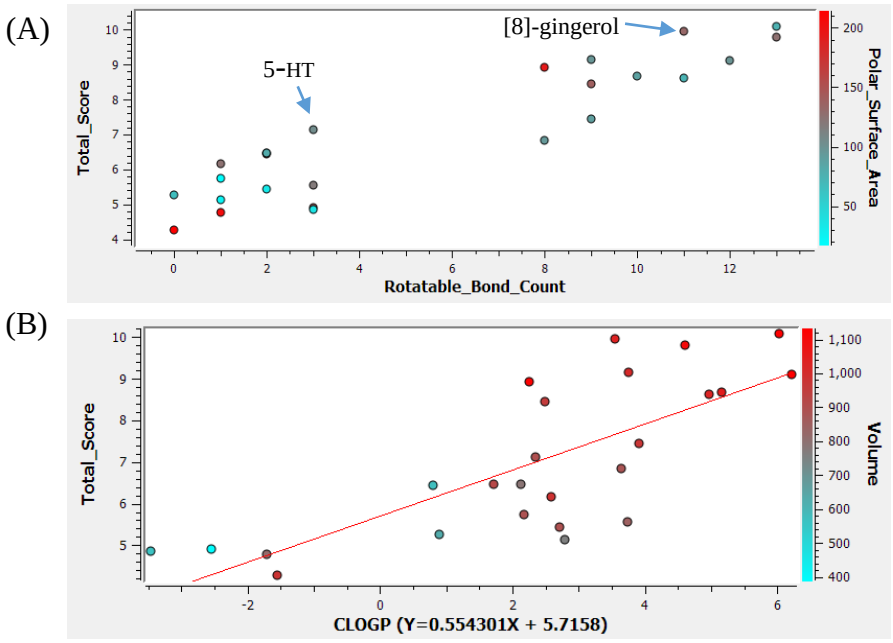


Figure 6-7. (A) Ligand rotatable bonds compared to total score and polar surface area. (B) Ligand clogP values versus total score and coloured by volume.

Unexpectedly, the ginger compounds scored highest in the serotonin binding site. In terms of drug-like characteristics, the ginger compounds have a high non-covalent interaction potential meaning that they possess a range of structural moieties required for maximizing binding affinity. These include an aromatic ring (pi stacking), alkyl tail (hydrophobic and Van der Waal interactions), hydrogen bond donors (phenol and hydroxyl) and acceptors (carbonyl and hydroxyl oxygens) for maximizing hydrogen bonding interactions. In addition, the ginger compounds have a high degree of flexibility as illustrated by the observed correlation between rotatable bonds and total score in Figure 6-7-A.

The same features are similarly found in the structural analogs, capsaicin and curcumin which also scored highly in the serotonin binding site. In addition to flexibility, there is a clear correlation between hydrophobic character and total score with all the top scoring ginger compounds and structural analogs having high, positive clogP values (Figure 6-7-B). Volume is similarly positively correlated with total scores (Figure 6-7-B).

The abilities of most current molecular docking algorithms to accurately model all factors present *in vivo* is still somewhat limited. Target flexibility, explicit solvent and some types of non-covalent interactions, for example, are often not considered or are dealt with poorly. As a result, caution in the interpretation of these results is required. Interactions between both competitive and non-competitive antagonists with the receptor have been described by Thompson et al.[2] to undergo a pathway as they progress from bound to unbound which may involve several transient sites. Furthermore, the nicotinic

receptor, AChBP, also of the CYS loop receptor is known to undergo substantial quaternary twisting of the subunit interface upon activation of the ion channel and bending of the extracellular domain.[15] It is feasible then to consider similar conformational movements of the 5-HT_3 receptor and concomitant changes to binding sites. In this light, it is not surprising that we see the ginger compounds binding well to this site. The crystal structure was assumed to have adopted a closed conformation upon binding of the inhibitory VHH nanobody.[13] It is possible that serotonin may have scored higher had the receptor been in a more open channel conformation and ligand ranking likely to be quite different.

Binding studies reveal a complexity in mechanisms of action with respect to how particular ligands may interact with different subunit stoichiometry. The potent inhibitor, Vuf1066 for example, was found to displace granisetron at the orthosteric binding site in an A+A- binding site but acted at an alternate site at an A+B- binding.[14] The $5HT_{3A}$ crystal structure used in this study is homomeric (A+A-).

Prior to the determination of the mouse crystal structure, site directed mutagenesis studies revealed a number of residues important for activation of the 5HT receptor or binding of serotonin.[16] These included Y46, F103, S136 and D138 (mouse numbering). These residues are more posterior to the serotonin site shown in the mouse structure and were not seen to interact with any of the ligands. In the crystal structure, however, Hassaine et al.[13] further identified several key residues in the crystal structure as important for serotonin binding. For example, we found that R65 played a key role in binding

ligands at the serotonin site and supports experimental observations. Several ginger compounds, structural analogs and competitive antagonists interacted with this residue through hydrogen bonding. In addition, N101 and T154 were also important for stabilizing ligands via hydrogen bonding. Serotonin was found to interact with D42 and D177 as well as S179. These residues were implicated by Hassaine et al.[13] as forming the serotonin binding site. The residues forming the most hydrogen bond interactions with the ligand database were R65, N101 and T154. Ginger ligands formed hydrogen bonds with several residues, predominantly R65, T154 and N101. We found previously unidentified residue, E102, contributed to stabilization of [10]-dehydroshogaol. The setron group of ligands docked into two main regions within the site. Ondasetron and dolasetron bound closer to the complementary site where 5-HT_3 was found to interact while granisetron and romasetron bound closer to the primary subunit face. Palonasetron was somewhere in between these regions (Figure 6-8A).

Y207 was identified as a residue able to add stability to ligand binding through pi stacking interactions observed with ginger ligands such as [8]-gingerol (Figure 6-9B). Other competitive antagonists interacted predominantly with R65 and T154; both of

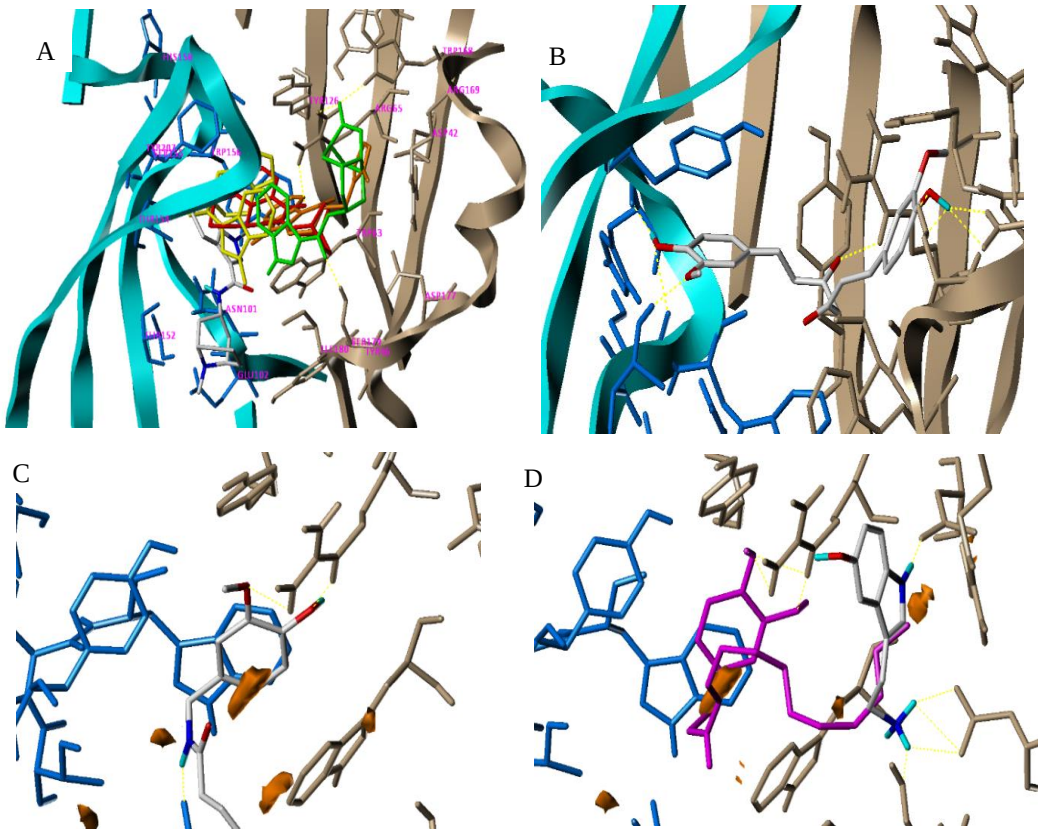


Figure 6-8 (A)-Granisetron (atom colours) ondasetron (orange) dolasetron (green) romasetron (yellow) palonasetron (red). (B) -Curcumin (atom colours) docked into serotonin site. Additional stability by possible pi stacking interaction with Y207. (C) Capsaicin docked into serotonin site depicting the aromatic box created by Y207, W156 (primary subunit, blue) andY126 & W63 (complementary subunit, beige). (D) Serotonin (atom colours) and [10]-G (magenta). Hydrophobic probe contoured at -1.5 kcal/mol for C and D

which also interacted with gingerols. Our docking results are in agreement with these residues as important for hydrogen bonding with the site (Table 6-1). Figure 8-C clearly depicts the aromatic box of the serotonin site created by the residues Y126, and W63 of the complementary subunit and Y207 and W156 of the primary subunit. The high scoring

ginger compounds and their structural analogs were observed to dock in a similar orientation with their aromatic ring embedded in this box permitting hydrogen bonding with the side chain cationic amine of R65. In contrast serotonin's aromatic ring appears to take advantage of a cation-pi interaction with R65 (Figure 6-8-D). This interaction was proposed by Hassaine et al.[13] with granisetron. We further purport that a second cation-pi interaction on the opposite face of serotonin's aromatic ring is possible with R169.

Of the ginger compounds, gingerols had the highest total score. A contributing factor towards the high score is likely to be the advantage taken of hydrogen bonding opportunities within the site. Both the shogaols and dehydroshogaols lack an alkyl hydroxyl group and have less flexibility due to the double bond. Due to their flexibility the length of their carbon chains did not negatively impact on their total scores. Non-competitive ligands and decoys, acetylcholine and GABA had the lowest score measured.

6.3.2 *Allosteric binding site*

Allosteric modulation facilitates fine tuning of ion permeation through the channel by signal dampening, for example, depending on the stoichiometry of the subunits and the number of serotonin ligands able to bind one receptor. Multiple modes of regulation have been noted in other CYS-loop receptors and is similarly likely in the serotonin receptor and involve a number of allosteric binding sites. Endogenous membrane lipids have been suggested to modulate ion permeation by binding to specific regions of the transmembrane channel. The exact location of binding for non-competitive antagonists of the $5HT_3$ receptor is yet to be fully elucidated. The transmembrane domain

of the CYS-loop receptors are functionally similar with key regions along the length of the ion permeation pathway being designated with a prime notation such that the pre-M2 region, identified as -20' contains a ring of cationic residues.[17] Certain residues of the M2 helix face in toward the channel (Figure 6-9) and site-directed mutagenesis studies have identified a number of residues important for channel function.

Furthermore non-competitive antagonists (NCAs) such as picrotoxin can differentiate different subunit compositions in the receptor.[18] The most likely position for

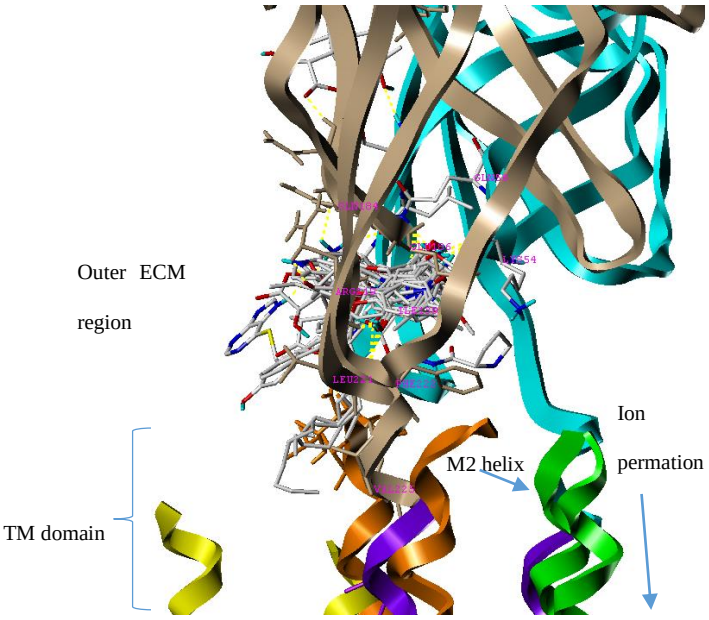


Figure 6-9. Docked ligands within the allosteric binding site at the ECM/TM interface between primary and complementary subunits. Side view with M2 TM helix (green)

many of the exogenous NCAs is the intersubunit interface at the top of the transmembrane domain. Another allosteric site is proposed in the pre-M2, ECM intersubunit interface. Anesthetics and small alcohols have been shown to interact at a similar site in GABA and glycine receptors.[19] These compounds illicit similar effects on the 5-HT_3 receptor.[12] Since

the structure of one such anesthetic, lidocaine, has a degree of structural similarity to serotonin and the ginger compounds, our study focused on the latter allosteric site.

The orientation of the docked ligands within the allosteric binding site docking experiment are depicted in Figure 6-10. Note that picrotoxin is bound to a unique site midway between the serotonin and allosteric sites. This ligand may bind to a different

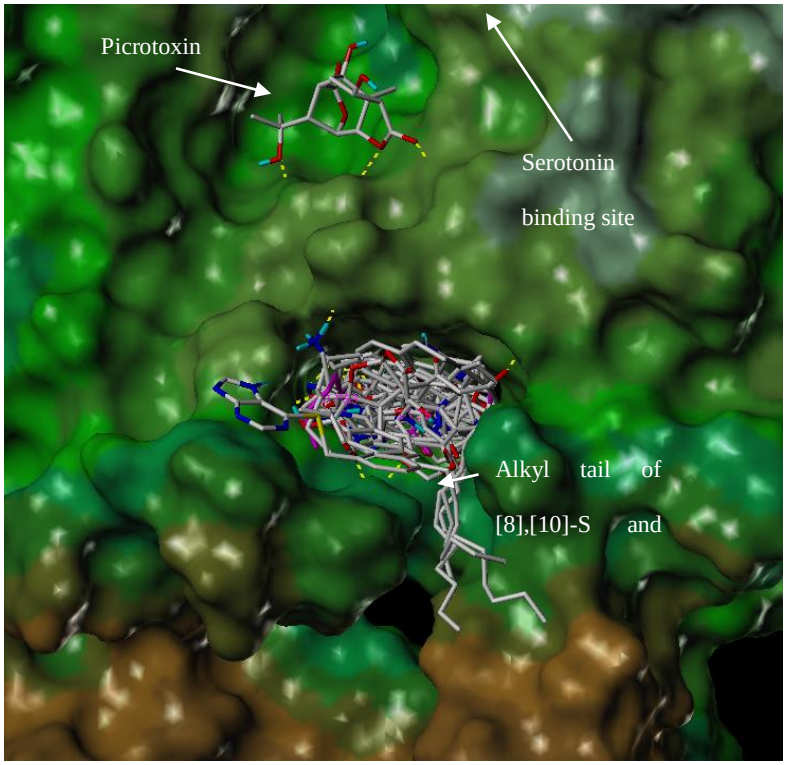


Figure 6-10 Superimposition of all ligands in allosteric site with 8G (magenta). Docked picrotoxin indicated position closer towards serotonin binding site. Alkyl tails of [8],[10]-S and [6],[10-DHSG. Surface contour coloured by lipophilic character (decreasing order of lipophilicity: brown, green, blue).

allosteric site with the A+B- subunit interface. The allosteric binding site depicted occupies a greater volume than that of the serotonin binding site enabling some ginger

compounds to adopt a more extended conformation. This appears to facilitate a favourable hydrophobic interaction between the alkyl moiety of these ligands with the hydrophobic region found closer to the transmembrane domain.

The ginger compounds and structural analogs also scored highest in this binding site with capsaicin attaining the highest total score. As demonstrated by the lower level of contouring for the hydrophobic probe, the allosteric site is more polar than the

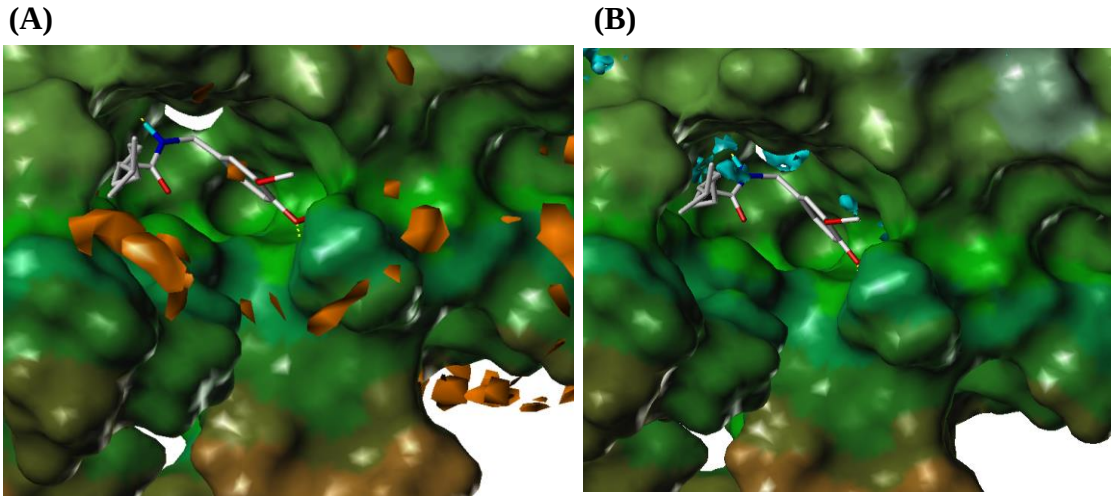


Figure 6-11. Allosteric site with highest scoring ligand, capsaicin.A: GRID contours for a hydrophobic probe (-0.5kcal/mol). B- GRID contours for a water probe (-11kcal/mol). Surface coloured by lipophilic character.

serotonin cavity although there are particular regions with hydrophobic character which correlate well with hydrophobic moieties of the ligands (Figure 6-11-A). Figure 6-11-B shows sites of strong interaction with a water probe which correlate well with the docked positions of polar groups on the ligands.

Comparison between the different ginger compounds showed the gingerols generally scoring higher as a group (Figure 6-12) with all three adopting a similar orientation within the site. This trend is continued with the shogaols scoring generally

higher than the dehydroshogaols. This trend correlates with the higher polarity of the gingerols compared to the other ginger compounds and, in this context, would therefore bind with higher affinity in a more polar site.

Serotonin and the competitive antagonists ranked moderately at this site with all setron ligands binding in a similar location to the gingerols (Figure 6-13-A and B). PU02 occupied a unique site lower down toward the transmembrane region forming a pi

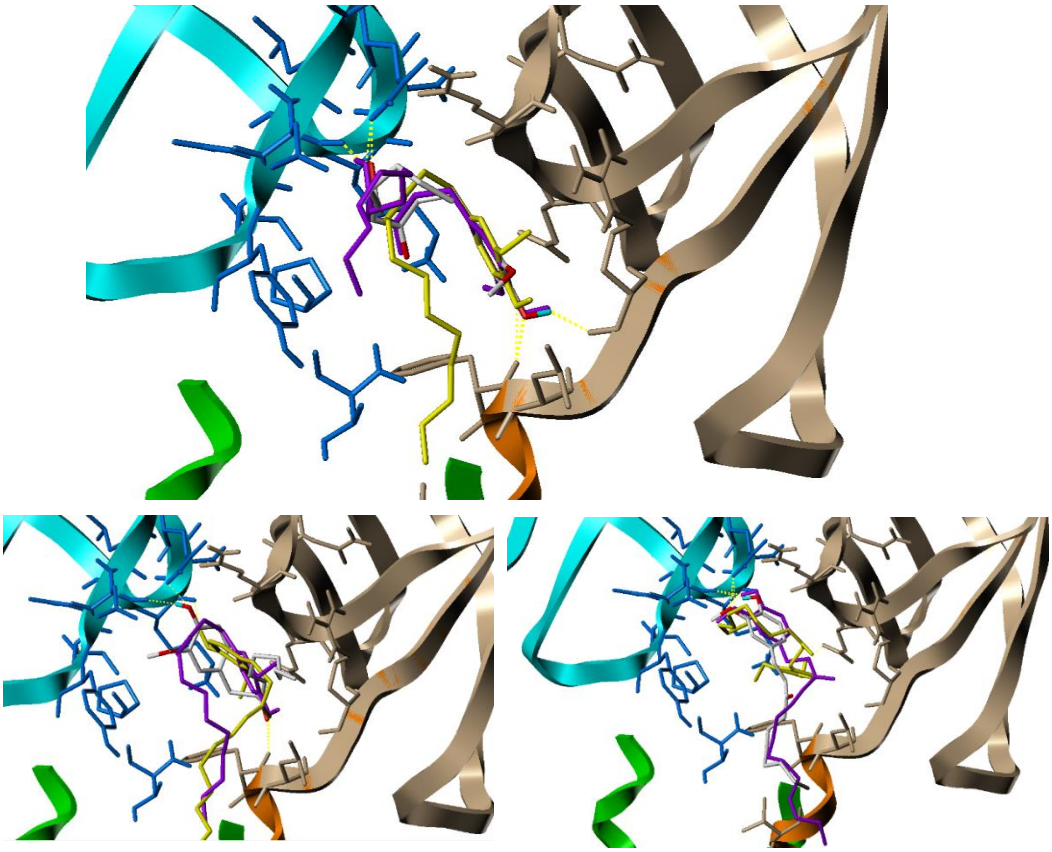


Figure 6-12 Superimposition of 6G (atom colours), 8G (yellow), 10G (violet) in allosteric site with key residues labelled. Primary subunit (cyan ribbon); Complementary subunit (beige ribbon)

stacking interaction with Y140. (Figure 6-13-C)

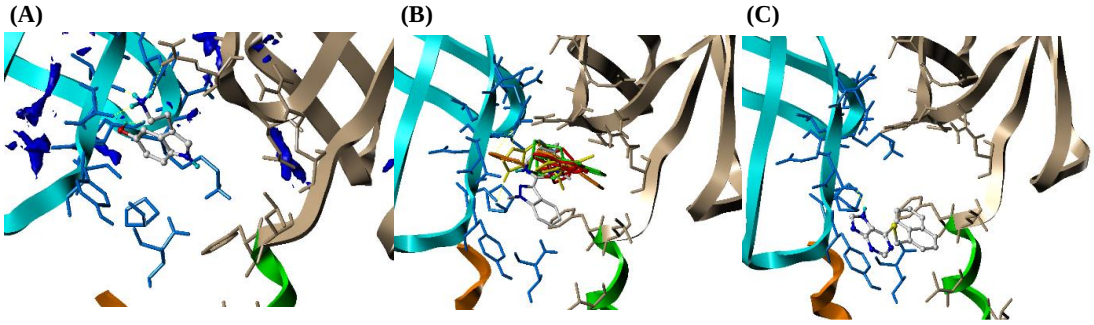


Figure 6-13 Allosteric site: (A)-Serotonin docked into allosteric site with amine cation probe contoured at -15kcal/mol (B) Setrons (granisetron (atom colours), ondasetron (orange), dolasetron (green), romasetron (yellow) palonosetron (red) (C)- PU02 docked into a unique orientation within allosteric site forming a pi

Our analysis identified R219, Q56, F222, E53, K54 and T280 as the key binding residues for this site with minor contributions from I139, P279 and E186. The key residues important for forming hydrogen bonds with the ginger compounds were I139, R219, Q56, F222, and Q53. F222, in particular, was involved with hydrogen bonding with all shogaols and most gingerols. Compared to serotonin and the ginger compounds, other competitive antagonists exhibited relatively low levels of hydrogen bonding interactions within the allosteric site suggesting less available hydrogen bond donors/acceptors at this site compared to the serotonin site. Flexibility played a positive role in how well the ligands scored at this site as it did within the serotonin site (Figure 6-14-A). Volume and increasing hydrophobicity were observed to play a similar role in contributing to a higher total score. (Figure 6-14-B).

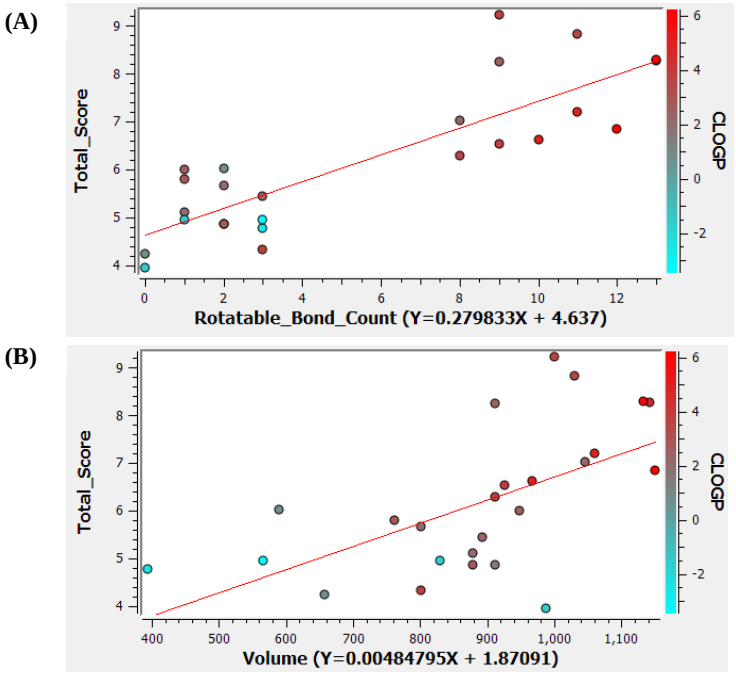


Figure 6-14. (A) Scatter plots of rotatble bonds Vs Total score with colour axis, clogP B) Scatter plots of Volume Vs Total score with colour axis, clogP

An alignment (see appendix) was performed between the mouse and human $5HT_3A$ and B receptors. Human and mouse A subunits share 84.7% sequence identity. Human and mouse B subunits share 73.2% sequence identity. Human A and B subunits share 44.75% identity whereas mouse A and B share 42.4% identity.

It was found that all key residues important for binding the ginger compounds (as well as serotonin) in the serotonin site were conserved between human and mouse A subunits. The newly identified E102 was similarly conserved. For the allosteric site, all residues noted as important for binding the ginger compounds were conserved between mouse and human sequences. Given the high degree of sequence similarity between the mouse and human subunits and the conservation of those important receptors, it is

unlikely that the species difference would account for the finding that the ginger compounds bound well in both sites.

6.4 Summary

In this study, we investigated the binding interactions of ginger [6], [8] and [10]-gingerol, [6], [8] and [10]-shogaol and [6], [8] and [10]-dehydroshogaol, as well as several known competitive and non-competitive antagonists at the orthosteric and allosteric binding sites on the 5-HT_3 receptor. Notably the ginger compounds scored highly at both sites along with the structural analogs, capsaicin and curcumin. It has been proposed that the ginger compounds elicit their effect through an allosteric modulation of the 5-HT_3 receptor. Our results support this hypothesis. Within the serotonin site, a high degree of hydrogen bonding and flexibility was proposed to contribute to their high score. Additionally, they also scored higher than other non-competitive antagonists at the allosteric site. Likewise, at the allosteric site, a high degree of hydrogen bonding and flexibility likely contributed to their overall high total scores (Table 6-1). The finding that the ginger compounds outscored serotonin and other competitive antagonists at the serotonin site may have a number of possible explanations.

In a saturation study performed by Walstab et al,[10] a competitive antagonist was present and likely bound at the orthosteric site. Under these conditions, due to their flexibility and relative non-specificity for a particular binding site, the ginger compounds may bind at the allosteric site to illicit their effect since they were unable to displace GR10655. A similar dual role has been observed for amide-type local anaesthetics, lidocaine and bupivacaine.[19] Structural characteristics of the ginger compounds which

could contribute to their capacity to bind well in different environments are their flexibility and combination of both a degree of polarity and hydrophobic character. These features could endow the compounds to take full advantage of the specific complementarity at each site.

Our results could reflect the structural changes that occur in the transition from open to closed channel conformations. Serotonin binds with high affinity to the open conformation. Hassaine et al,[13] speculated that the crystal structure they produced is in the closed conformation. Thus it is possible that the overall score of serotonin was lower than what may have been observed for the open conformation. Additionally, the crystal structure depicts a A+A- subunit homomeric structure. Given the five currently identified subunits, varying degrees of binding affinity would be expected by all ligands with the concomitant changes to the binding site. Allosteric modulators are more potent in the heteromeric receptors. To test this idea in the absence of another crystal structure, work is in progress to prepare a homology model of an A+B- and a B-A+ receptor using a three dimensional template from the current 5-HT_3 receptor.

To date, no 5-HT_3 crystal structures exists with a ligand bound to either the serotonin binding or the allosteric site 5-HT_3 receptor. High resolution, three dimensional structures of other cation selective CYS loop receptors, such as the nicotinic acetylcholine receptor have been published, which share a high degree of similarity to the 5-HT_3 receptor. It is likely that they share a degree of functional similarity as well. While we investigated the two key sites identified to date in this study, it is also possible that

additional binding sites for allosteric regulation exist. Future studies could explore other areas of the receptor such as the transmembrane region.

We also acknowledge the following limitations. Only one crystal structure of the 5-HT_3 receptor is currently available and while this allows for *in silico* investigation of this receptor, the crystal structure is not highly resolved making positioning of target atoms/side chains difficult. Since rigid docking approaches of this kind relies on the accurate position of the sidechains, this will restrict the ability to test conformation space sufficiently well to find the most realistic binding poses. The effects of the low resolution X-ray imaging has been somewhat reduced by conducting energy minimisation on the target prior to docking to relieve any initial strain in the conformation of the protein although gross misplacement of atoms/residues side-chains will not be compensated for by this measure. Since some of the key binding residues have long, flexible side chains (R219, K54, R65) and thus have a high degree of mobility, docking algorithms incorporating more flexible approached would be preferable. In addition, quantum mechanical molecular dynamics simulations incorporating explicit solvent could also offer improved results.

6.5 Experimental procedures

All modelling work was performed using SYBYL-X version 2.1.[20]

6.5.1 *Target and ligand library preparation*

One principle and one complimentary subunit of the 5-HT_3 receptor were extracted. Hydrogens were added and Gasteiger-Huckel charges were assigned to the

atoms of each compound prior to energy minimization (Amber FF99) to a convergence of 0.5 kcal per mol. Extraneous ligands were removed prior to docking.

Known competitive antagonists, structural analogs to gingerols, non-competitive antagonists and decoys (molecules known not to bind to the 5-HT_3 receptor) were included in the analysis in order to compare binding characteristics with the ginger compounds.

6.5.2 *Docking*

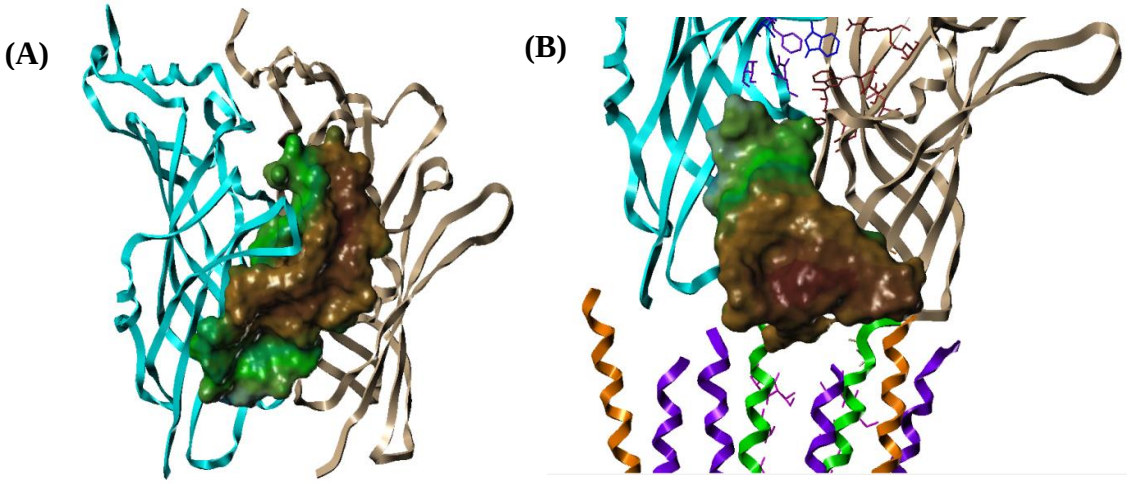


Figure 6-15 The protomol of the (A) serotonin binding site and (B) the allosteric binding site

The Surflex-Dock 2.1 algorithm, based on a multi-channel protomol generational methodology, was employed for the serotonin binding site while a residue-based protomol was used for the protomol generation at the allosteric binding site. The protomol at each serotonin and allosteric site was generated using a threshold value of 0.5 and 0.9 and a bloat of 10 and 10, respectively to create a protomol which sufficiently covered the site of interest.

Figure 6-15 depicts the size and orientation of the protomol for the serotonin and allosteric binding sites respectively within which the set of 25 ligands were docked. The protomol was checked to ensure all ligands were included therein. Consensus scoring (C_{score}) was included to identify structures obtaining high scores across all 4 scoring functions. C_{scores} are between 6-5. A C_{score} of 5 reflects complete consensus of the pose binding score across all scoring functions.

6.5.3 *GRID Contouring*

Peter Goodford's program, Grid,[21] was employed to discover sites of potentially strong binding interaction between a target and probe. A number of single atom and multi-atom probes were selected to best reflect the functional group characteristics of serotonin and the ligands of interest. A box of dimensions (topx,y,z; botx,y,z) was generated around the two sites of interest on the receptor (*see appendix*). The resolution (number of grid points at which to calculate the interaction energy between probe and target) was set to (0.33Å). The LEAU parameter was set to 1 where the probe contained 2 or more hydrogen bond donor/acceptors, otherwise it was 0. Other settings were left at default values. The following probes were used: water (OH2), aromatic carbon (C1=), methyl carbon (C3), phenolic hydroxyl oxygen (O1), alkyl hydroxyl oxygen (OH), carbonyl oxygen O, hydrophilic (DRY) and amphipathic (BOTH).

6.6 References

1. Hesketh P. Chemotherapy-induced nausea and vomiting. *N Engl J Med.* 2008;358:2482 - 2494.
2. Thompson AJ, Lummis SCR. 5-HT(3) Receptors. *Current pharmaceutical design.* 2006;12(28):3615-3630.
3. Nelson DR, Thomas DR. [3H]-BRL 43694 (Granisetron), a specific ligand for 5-HT3 binding sites in rat brain cortical membranes. *Biochemical pharmacology.* 1989;38(10):1693-1695.
4. Downie DL, Hope AG, Belelli D, et al. The interaction of trichloroethanol with murine recombinant 5-HT3 receptors. *British Journal of Pharmacology.* 1995;114(8):1641-1651.
5. Marx W, Kiss N, Isenring L. Is ginger beneficial for nausea and vomiting? An update of the literature. *Current Opinion in Supportive and Palliative Care.* 2015;9(2):189-195.
6. Marx WM, Teleni L, McCarthy AL, et al. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. *Nutr. Rev.* 2013;71(4):245-254.
7. Bhattarai S, Tran VH, Duke CC. The stability of gingerol and shogaol in aqueous solutions. *J Pharm Sci.* 2001;90(10):1658-1664.

8. Tian-Shung W, You-Cheng W, Pei-Lin W, Ching-Yuh C, Yann-Lii L, Yu-Yi C. Structure and synthesis of [n]-dehydroshogaols from Zingiber officinale. *Phytochemistry.* 1998;48(5):889-891.

9. Abdel-Aziz H, Windeck T, Ploch M, Verspohl E. Mode of action of gingerols and shogaols on 5-HT3 receptors: binding studies, cation uptake by the receptor channel and contraction of isolated guinea-pig ileum. *Eur J Pharmacol.* 2006;530:136 - 143.

10. Walstab J, Kruger D, Stark T, et al. Ginger and its pungent constituents non-competitively inhibit activation of human recombinant and native 5-HT3 receptors of enteric neurons. *Neurogastroenterol Motil.* 2013;25(5):439-447, e302.

11. Marx W, Ried K, McCarthy AL, et al. Ginger-Mechanism of Action in Chemotherapy-induced Nausea and Vomiting: A Review. *Crit Rev Food Sci Nutr.* 2015:0.

12. Davies PA. Allosteric modulation of the 5-HT3 receptor. *Current opinion in pharmacology.* 2011;11(1):75-80.

13. Hassaine G, Deluz C, Grasso L, et al. X-ray structure of the mouse serotonin 5-HT3 receptor. *Nature.* 2014;512(7514):276-281.

14. Trattnig SM, Harpsoe K, Thygesen SB, et al. Discovery of a novel allosteric modulator of 5-HT3 receptors: inhibition and potentiation of Cys-loop receptor signaling through a conserved transmembrane intersubunit site. *The Journal of biological chemistry.* 2012;287(30):25241-25254.

15. Taly A, Corringer PJ, Guedin D, Lestage P, Changeux JP. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. *Nature reviews. Drug discovery.* 2009;8(9):733-750.

16. Del Cadia M, De Rienzo F, Weston DA, Thompson AJ, Menziani MC, Lummis SC. Exploring a potential palonosetron allosteric binding site in the 5-HT(3) receptor. *Bioorganic & medicinal chemistry.* 2013;21(23):7523-7528.

17. Thompson AJ, Lester HA, Lummis SC. The structural basis of function in Cys-loop receptors. *Quarterly reviews of biophysics.* 2010;43(4):449-499.

18. Thompson AJ. Recent developments in 5-HT3 receptor pharmacology. *Trends in pharmacological sciences.* 2013;34(2):100-109.

19. Ueta K, Suzuki T, Sugimoto M, Uchida I, Mashimo T. Local anesthetics have different mechanisms and sites of action at recombinant 5-HT3 receptors. *Reg Anesth Pain Med.* 2007;32(6):462-470.

20. *SYBYL-X 2.1* [computer program]. 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.: Tripos International.

21. Goodford PJ. A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. *Journal of medicinal chemistry.* 1985;28(7):849-857.

Chapter 7. The concentration of major active constituents within commercial ginger products using reverse phase-high performance liquid chromatography

The manuscript included in this chapter analysed the concentration of bioactive compounds within twenty commercial ginger products using Reverse Phase-High Performance Liquid Chromatography. The results have significant implications for both future clinical trial design and clinical practice.

Citation: Marx WM, Isenring E, Schweiker S, McCarthy AL, Ried K, Sali A, Vitetta L, Lohning A. The concentration of major active constituents within commercial ginger products using reverse phase-high performance liquid chromatography. Journal of Chromatography A. Impact factor: 4.298; Intended submission: December, 2015

7.1 Abstract

Background: The rhizome of ginger (*Zingiber officinale*) contains many bioactive compounds, primarily gingerols and their degradation products, shogaols. Studies suggest that these compounds could exert a beneficial effect on the symptoms of several chronic conditions (e.g. diabetes, arthritis) and in the reduction of nausea associated with morning and motion sickness, and chemotherapy. However, it is unknown if ginger supplements and food products contain sufficient quantities of these active ingredients to achieve a therapeutic effect.

Aim: The aim of this study was to determine the concentration of [6]- [8]- and [10]- gingerol and [6]- and [10]-shogaol within 20 commercially available ginger products including ginger dietary supplements, ginger spices (ground dried ginger) and ginger-containing drinks and food products.

Method: Samples were extracted prior to separation by reverse phase-high performance liquid chromatography. UV detection was conducted at 205nm. Component peaks were identified by retention time of a set of standards.

Results: Per gram, ginger supplements, particularly the standardized extracts, contained the greatest concentration of measured compounds (2.60±1.38 mg), while the concentration of compounds within spices (1.86± 1.35 mg), beverages (0.32± 0.21 mg), confectionary (0.09± 0.07 mg), and teas (0.03± 0.0002 mg) was considerably lower. When the concentration of compounds was measured per standardised serve, four ginger

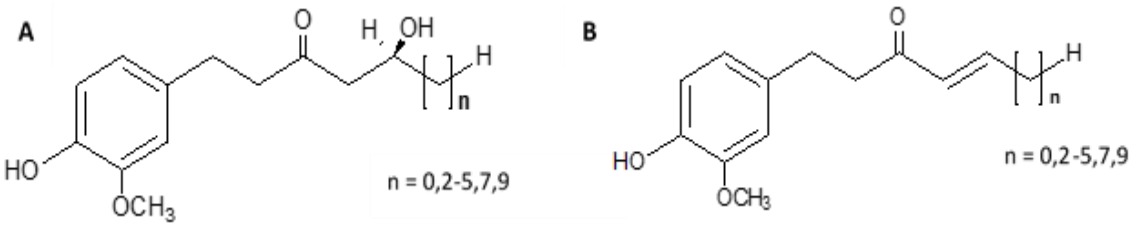
confectionary and beverage products contained total gingerol and shogaol concentrations that were similar to the analyzed dietary supplement.

Conclusion: The results of this study demonstrate that per standardised serve, certain beverages and confectionary contained similar or greater concentrations of the primary bioactive compounds of ginger compared to a selection of supplements. Future clinical trials investigating ginger should ensure that the consumption of other ginger products are monitored so as to avoid confounding results. In addition, inconsistent concentrations of active compounds within various ginger supplements demonstrate the need for standardized extracts with concentrations that have been confirmed using technologies such as high performance liquid chromatography prior to undertaking clinical trials of ginger to ensure sufficiently potent interventions.

7.2 Introduction

The rhizome of ginger (*Zingiber officinale*) contains many bioactive compounds. The gingerol class of compounds is the primary bioactive element within the non-volatile, pungent component of ginger. Shogaols are the degradation product of gingerols. These are present in low concentrations in fresh ginger but increase when gingerols are exposed to heat, due to the presence of the β-hydroxy ketone group in the gingerol structure.[1] The shogaols and gingerols are differentiated by the presence of either a hydroxyl group on the alkyl chain (gingerols) or oxidation of the hydroxyl group for a double bond (shogaols, Figure 7-1). These compounds have been studied in clinical and pre-clinical studies for their effect on several chronic conditions (e.g. diabetes and arthritis). [2-4] The potentially beneficial effect of ginger on nausea has also been an area of significant research interest. A growing body of clinical trials has provided preliminary support for its use in multiple types of nausea such as motion sickness, morning sickness and chemotherapy-induced nausea and vomiting.[5-7] Studies that have investigated the antioxidant, anti-inflammatory, and chemo-preventive effect of individual compounds contained in ginger have reported different levels of activity depending on the chain length and presence of an alpha, beta-unsaturated ketone group. For example, when several gingerol and shogaol compounds were compared, Dugasini et al.[8] reported [6]-shogaol to be the most potent inhibitor of inflammation and reactive oxygen species production and [10]-gingerol to be the most potent gingerol.

Figure 7-1 Chemical structures of gingerols (A) and shogaols (B).



A, n = 0, 2, 4 corresponds to [6]-, [8]- and [10]- gingerol. In image B, n = 0, 4 corresponds to [6] and [10]- shogaol.

Because of purported medicinal effects, ginger products are often used by the general population as complementary medicines and are sometimes recommended by healthcare professionals as adjuvants to standard therapy.[9-11] However, there are currently few studies that have investigated the concentration of active compounds in commercially available products, thereby providing a reliable guide to appropriate use of these products.[12,13] Due to the increasing public use of complementary treatments such as dietary supplements, information regarding the potency of available ginger preparations will also be of interest to healthcare professionals seeking these products for their adjuvant medicinal properties to determine their potential to produce side effects and interactions. Due to the differing biological activity of the gingerol and shogaol compounds, it is also prudent to measure the concentration of each of these individual compounds within widely available ginger products.

The aim of this study was to determine the concentration of [6]-, [8]- and [10]-gingerol and [6]- and [10]-shogaol within commercially available ginger products, including dietary supplements and ginger-containing drinks and food products using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC).

7.3 Methods

7.3.1 *Chemicals and Materials*

HPLC grade water, methanol, ethyl acetate, and acetonitrile were purchased from ThermoFisher (Massachusetts, USA) and Sigma Aldrich (Missouri, USA). [6]- [8]- and [10]- gingerol and [6]- and [10]-shogaol standards (Table 7-1) were purchased from Chromadex (Irvine, CA, U.S.A). Ginger products were purchased from one local supermarket (Gold Coast, Australia) and one online store (based in New Zealand) in April, 2014. In addition, one supplement was supplied by the respective manufacturer for use in this study. In total, 20 products were purchased, including dietary supplements, beverages, spices (ground ginger), teas, and confectionary.

Table 7-1 Physical properties of analyzed compounds

Compound	IUPAC nomenclature	Chemical Formula	MW	cLogP*
[6]-gingerol	(5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	$C_{17}H_{26}O_4$	292.37	2.489
[8]-gingerol	(5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-dodecanone	C19H30O4	320.42	3.547
[6]-shogaol	(E)-1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one	$C_{17}H_{24}O_3$	276.37	3.811
[10]-gingerol	(5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-tetradecanone	$C_{21}H_{34}O_4$	348.48	4.605
[10]-shogaol	(E)-1-(4-hydroxy-3-methoxyphenyl)tetradec-4-en-3-one	$C_{21}H_{32}O_3$	322.48	6.027

(*Calculated logP obtained from SYBYLX-2.1)

7.3.2 *Sample preparation*

Due to the variety of types of ginger products analyzed, two extraction protocols were required. All samples were prepared in triplicate. Percentage yield from each extraction protocol was determined by conducting each extraction protocol with a 0.08 mg/ml standard mix.

7.3.3 *Ethyl acetate extraction*

In order to analyze the ginger supplements, biscuit, and spices, ethyl acetate (10mL) was added to 500mg samples of spices, pierced supplements or crushed biscuit.

Samples were vortexed then sonicated for 30 minutes using a CamLab TransSonic T310 sonicator. Samples were centrifuged (20 minutes at 2500rpm and 25 degrees Celsius) using a Beckman Coulter Allegra X-15R centrifuge. The supernatant was subjected to second pass extraction using an additional ethyl acetate (10 mL). The supernatants of both extractions from each product were combined and evaporated to dryness. The samples were reconstituted in methanol (1.5ml) and stored at 4ºC.

Serving sizes for the supplements and biscuit were in accordance with suggested serving sizes by the manufacturer. The serving size for the spices was set at one gram.

7.3.4 *Liquid/liquid extraction*

Beverage samples (50ml, degassed) and confectionary samples (500mg) were diluted in HPLC water (15ml). For the tea products, three tea bags were infused in HPLC-grade water (50ml, room temperature) for three minutes. All samples were left overnight

and then extracted in ethyl acetate (10ml). A second pass extraction was conducted. The supernatants of both extractions from each product were combined and evaporated to dryness. The samples were reconstituted in methanol (1.5ml) and stored at 4ºC.

The serving size of the beverages was set at 250ml and confectionary serving size was defined as 5g, as this was found to be the approximate weight of a single piece of confectionary.

7.3.5 *Standard preparation*

Stock solutions (10mL) of each standard were prepared from the 5mg material supplied by the manufacturer Chromadex (Irvine, CA, U.S.A). A 0.4 mg/mL stock solution that contained each standard was prepared. A dilution series of the standard mix were prepared as needed between 0.50 ug/mL to 200 ug/mL. Working standards were prepared in the range of 0.0005 to 0.2 mg/mL and stored at 4ºC.

7.3.6 *HPLC analysis*

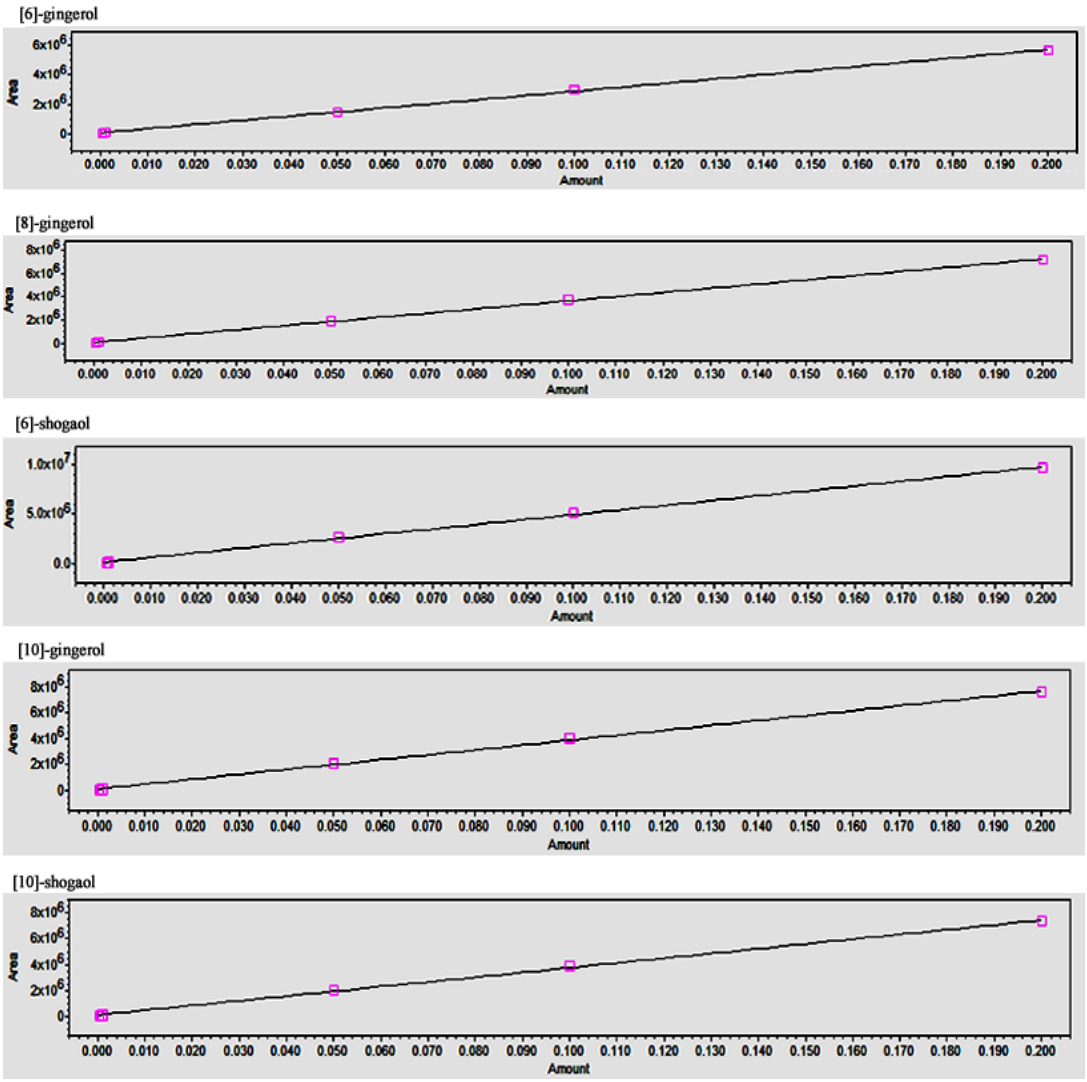
Ginger samples were separated on a Waters Alliance e-2695 Separations System RP-HPLC and detected with a 2489 Dual-Beam UV detector. A 150x4.6mm C-18 reversed phase column (Luna C18 5µM; Phenomenex, USA) was fitted with a guard column.

The mobile phase consisted of HPLC-grade water (A) and acetonitrile (B) at starting conditions of 90% A. Analytical conditions included an injection volume of 10µL, flow rate of 1.5ml per minute and a column temperature of 27ºC. A binary gradient elution system was applied as follows: 0.0–1.85 min, 10–50% B; 1.86–7.88 min, 55% B;

7.89–11.59 min, 66% B; 12–17.6 min, 100% B. 17.61-25 min 10% B. The ultraviolet absorbance was measured at 205nm. Peak identification was based on the retention time of the standards.

7.4 Results

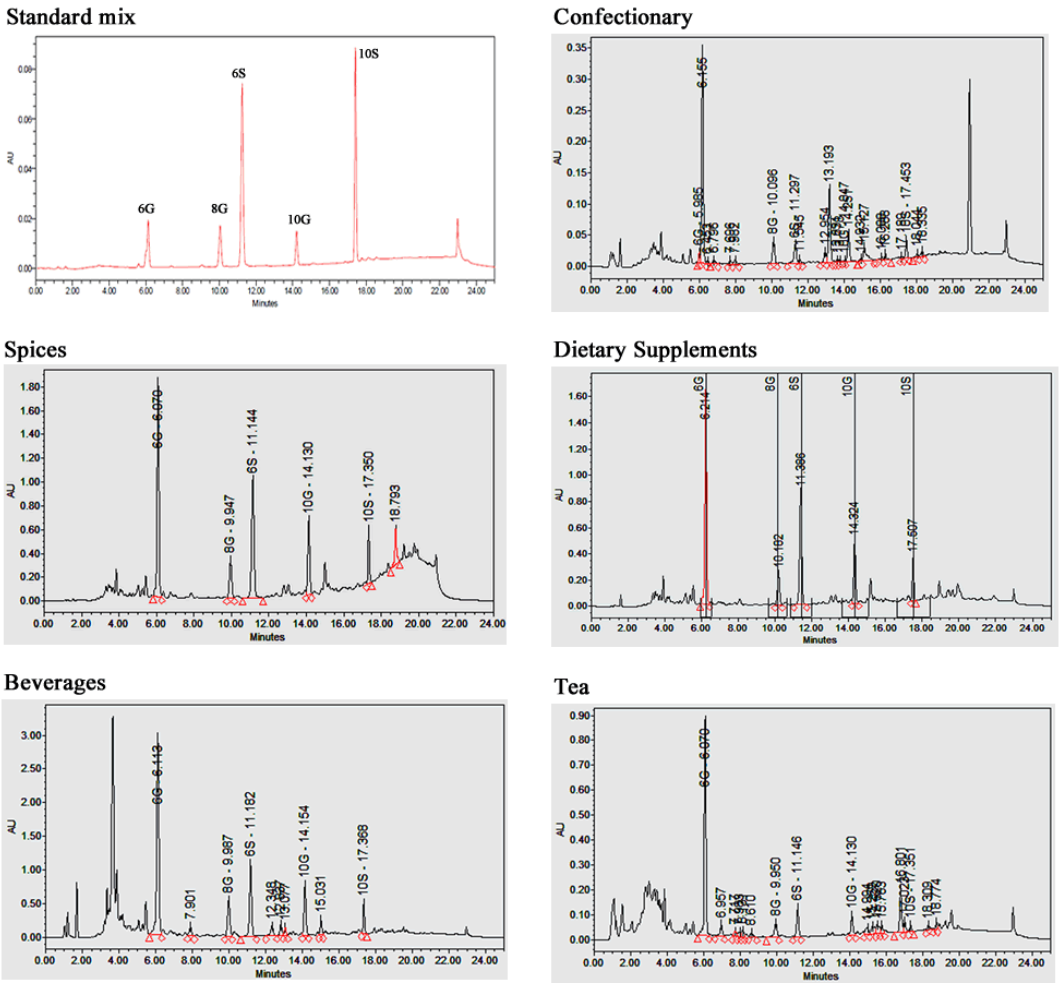
Figure 7-2 Standard curves of each analyzed compound



The standard curves for [6]-gingerol, [6]-shogaol, [8]-gingerol, [10]-shogaol, and [10]-gingerol (Figure 7-2) were linear from 10.0 to 1000.0 g/mL (correlation coefficient for each compound were ≥0.9992).

Figure 7-3 depicts a sample chromatogram for the standard mix and a sample from each product category. In accordance with the lipophilicity of each compound (Table 7-1), the elution order for analysis was: [6]-gingerol, [8]-gingerol, [6]-shogaol, [10]-gingerol, [10]-shogaol.

Figure 7-3 Sample chromatogram from each product category and standard mix



The results demonstrated that the liquid/liquid extraction procedure provided a greater yield when compared to the ethyl acetate extraction procedure for all compounds except for [6]-gingerol (Table 7-2).

Table 7-2 Percentage yield of liquid/liquid and ethyl acetate extraction procedure

	6G (%)	8G (%)	6S (%)	10G (%)	10S (%)
Liquid/Liquid extraction	25	74	76	59	74
Ethyl acetate extraction	32	33	33	28	37

The mean concentration of [6]-gingerol, [6]-shogaol, [8]-gingerol, [10]-gingerol and [10]-shogaol of all ginger products are tabulated per gram (Table 7-3) and per serving (Table 7-4) after they had been adjusted by the percentage yield as determined by the validation protocol

In all samples, [6]-gingerol was consistently detected in the highest concentration when compared to all other compounds investigated while [8]-gingerol and [10]-shogaol were found in the lowest concentration. In descending order, the total concentration of each compound from all analyzed products was [6]-gingerol (67.420mg), [6]-shogaol (20.175mg), [10]-gingerol (15.517mg), [10]-shogaol (7.784mg), [8]-gingerol (7.575mg). This order of [6]-gingerol, [6]-shogaol, and [10]-gingerol remained consistent for each product; however, some products contained slightly higher concentrations of [8]-gingerol than [10]-shogaol.

.

Table 7-3. Mean ± standard deviation concentration of [6]-gingerol, [6]-shogaol, [8]-gingerol, [10]-gingerol and [10]-shogaol in analyzed products per gram.

Supplements	Total (mg/capsule)	6-gingerol (mg/L)	8-gingerol (mg/L)	10-gingerol (mg/L)	6-shogaol (mg/L)	10-shogaol (mg/L)
Nature's Own Travel Well 1000	0.66	0.47±0.02	0.00±0.00	0.02±0.01	0.14±0.01	0.03±0.00
Lifestream Ginger capsules	7.44	4.59±0.73	0.35±0.20	0.88±0.15	1.22±0.21	0.38±0.07
Nature's Sunshine Ginger	7.02	2.32±0.03	0.43±0.02	0.89±0.02	2.20±0.05	1.17±0.04
BlueBonnet Ginger root extract	23.57	12.76±0.01	1.74±0.01	2.89±0.01	4.63±0.01	1.55±0.01
Blackmores Travel Calm Ginger	7.36	4.35±0.15	0.61±0.02	1.00±0.04	1.03±0.03	0.37±0.02
Helsinn Integrative Care Ginpax 40mg soft gel capsule	14.46	11.08±1.49	0.14±0.04	2.77±0.44	0.02±0.01	0.44±0.05
Spices	**Total (mg/g)**	**6-gingerol (mg/L)**	**8-gingerol (mg/L)**	**10-gingerol (mg/L)**	**6-shogaol (mg/L)**	**10-shogaol (mg/L)**
Woolworth's Ginger Spice Powder	13.42	5.64±0.02	1.34±0.00	3.11±0	2.13±0.00	1.19±0.0
Coles Ginger Spice Powder	12.93	5.50±0.17	1.21±0.04	3.12±0.1	2.01±0.0	1.09±0.04
Hertz Ginger spice	1.53	0.88±0.13	0.14±0.02	0.22±0	0.21±0.03	0.08±0.01
Beverages	**Total (mg/L)**	**6-gingerol (mg/L)**	**8-gingerol (mg/L)**	**10-gingerol (mg/L)**	**6-shogaol (mg/L)**	**10-shogaol (mg/L)**
Coles Dry Ginger Ale	1.94	1.58±0.66	0.04±0	0.18±0.1	0.10±0.08	0.04±0.04
Bundaberg Diet Ginger Beer	0.61	0.53±0.02	0.02±0.00	0.00±0	0.05±0.0	0.00±0.0
Saxby Dry Ginger Beer	2.20	1.85±0.21	0.04±0.03	0.14±0	0.11±0.01	0.06±0.01

Cascade Ginger Beer	3.23	2.86±0.41	0.07±0.04	0.13±0	0.12±0.01	0.04±0.0
Tiger Ginger Beer	0.86	0.50±0.66	0.04±0.03	0.18±0.1	0.10±0.08	0.04±0.04
Confectionary	**Total (mg/L)**	**6-gingerol (mg/L)**	**8-gingerol (mg/L)**	**10-gingerol (mg/L)**	**6-shogaol (mg/L)**	**10-shogaol (mg/L)**
Gin Gins Candies	0.68	0.60±0.05	0.02±0.00	0.02±0	0.03±0.0	0.01±0.0
Buderim Crystallised Ginger	0.70	0.63±0.06	0.00±0.00	0.02±0	0.04±0.0	0.00±0.0
Buderim Ginger bears	0.34	0.32±0.02	0.01±0.00	0.00±0	0.01±0.00	0.00±0
Arnotts GingerNut Biscuits	0.02	0.02±0.00	0.00±0.00	0.00±0	0.00±0	0.00±0
Teas	**Total (mg/L)**	**6-gingerol (mg/L)**	**8-gingerol (mg/L)**	**10-gingerol (mg/L)**	**6-shogaol (mg/L)**	**10-shogaol (mg/L)**
Twinings Ginger & Lemon Tea	0.13	0.13±0.00	0.00±0.00	0.00±0	0.00±0	0.00±0
Nerada Ginger & Lemon Tea	0.13	0.13±0.01	0.00±0.00	0.00±0	0.00±0	0.00±0

The concentration of analyzed compounds, particularly within the beverages, varied considerably (Figure 7-4). When compared per gram, with one exception, ginger supplements (particularly those that were made from standardized extracts) contained the largest concentration of analyzed compounds when compared to all other ginger products. Ginger spices also tended to contain a large concentration of compounds per gram while the ginger biscuit contained the lowest amount. When the concentration of each product was compared per serve, standardized extracts still contained some of the largest concentration of compounds. Excluding the tea samples, products from other categories (i.e. beverages, spices, and confectionary) contained concentrations of compounds similar to the standardized extracts.

Figure 7-4 Total mean gingerol and shogaol content of ginger products per gram

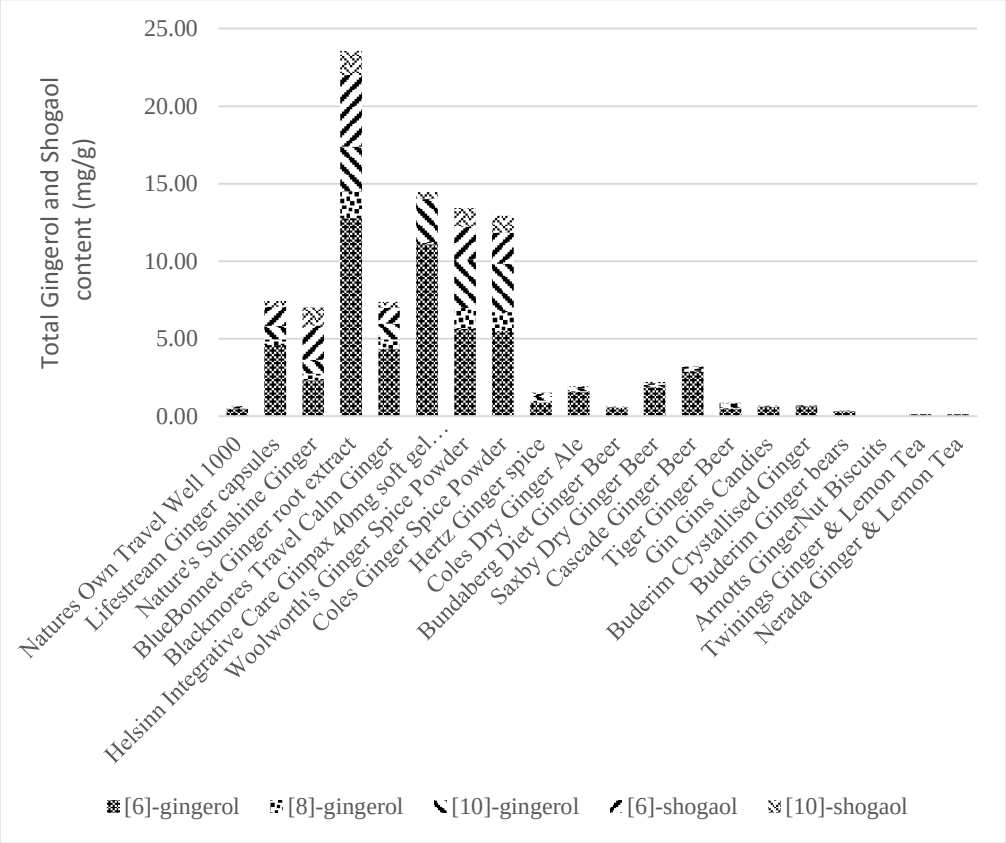


Table 7-4 Mean ± standard deviation concentration of [6]-gingerol, [6]-shogaol, [8]-gingerol, [10]-gingerol and [10]-shogaol in analyzed products per serving size.

Supplements	Estimated serving size (g)	Total (Per serving)	6-gingerol (Per serving)	8-gingerol (Per serving)	10-gingerol (Per serving)	6-shogaol (Per serving)	10-shogaol (Per serving)
Nature's Own Travel Well 1000	0.85g	1.68	1.19±0.05	0.00±0	0.36±0.03	0.05±0.026	0.08±0
Lifestream Ginger capsules	0.66g	18.92	11.68±1.86	0.90±0.51	3.11±0.53	2.25±0.381	0.98±0.17
Nature's Sunshine Ginger	0.60g	12.58	4.16±0.05	0.76±0.03	3.95±0.1	1.60±0.037	2.10±0.07
BlueBonnet Ginger root extract	0.52g	36.43	19.72±0.02	2.69±0.02	7.15±0.02	4.46±0.016	2.39±0.02
Blackmores Travel Calm Ginger	0.51g	11.16	6.60±0.22	0.92±0.03	1.56±0.05	1.51±0.055	0.57±0.03
Helsinn Integrative Care Ginpax 40mg soft gel capsule	0.24g	21.68	16.63±2.23	0.21±0.05	0.03±0.02	4.16±0.665	0.65±0.08
Spices		**Total (Per serving)**	**6-gingerol (Per serving)**	**8-gingerol (Per serving)**	**10-gingerol (Per serving)**	**6-shogaol (Per serving)**	**10-shogaol (Per serving)**
Woolworth's Ginger Spice Powder	1g	40.25	16.92±0.05	4.03±0	9.34±0	6.39±0.007	3.57±0.01
Coles Ginger Spice Powder	1g	38.80	16.50±0.52	3.64±0.12	9.35±0.26	6.04±0.178	3.28±0.11
Hertz Ginger spice	1g	4.58	2.64±0.38	0.41±0.06	0.67±0.1	0.63±0.103	0.23±0.04
Beverages		**Total (Per serving)**	**6-gingerol (Per serving)**	**8-gingerol (Per serving)**	**10-gingerol (Per serving)**	**6-shogaol (Per serving)**	**10-shogaol (Per serving)**
Coles Dry Ginger Ale	250ml	4.56	3.98±0.13	0.18±0.01	0.03±0	0.35±0.016	0.01±0
Bundaberg Diet Ginger Beer	250ml	16.50	13.89±1.61	0.31±0.2	1.02±0.12	0.80±0.083	0.47±0.06
Saxby Dry Ginger Beer	250ml	24.23	21.49±3.05	0.51±0.31	1.01±0.13	0.90±0.077	0.32±0.03

Cascade Ginger Beer	250ml	6.48	3.76±4.98	0.30±0.24	1.32±0.89	0.77±0.61	0.33±0.28
Tiger Ginger Beer	250ml	5.09	4.48±0.41	0.14±0.02	0.18±0.02	0.22±0.037	0.07±0.02
Confectionary		**Total (Per serving)**	**6-gingerol (Per serving)**	**8-gingerol (Per serving)**	**10-gingerol (Per serving)**	**6-shogaol (Per serving)**	**10-shogaol (Per serving)**
Gin Gins Candies	12g	25.12	22.85±2.20	0.15±0.03	0.65±0.07	1.40±0.141	0.08±0.02
Buderim Crystallised Ginger	25g	5.16	4.74±0.25	0.16±0.01	0.00±0.00	0.19±0.008	0.06±0
Buderim Ginger bears	48g	11.88	10.38±0.84	0.58±0.02	0.45±0.02	0.43±0.025	0.04±0
Arnotts GingerNut Biscuits	1 biscuit	2.14	1.28±0.03	0.14±0.00	0.29±0.00	0.24±0	0.19±0.03
Teas		**Total (Per serving)**	**6-gingerol (Per serving)**	**8-gingerol (Per serving)**	**10-gingerol (Per serving)**	**6-shogaol (Per serving)**	**10-shogaol (Per serving)**
Twinings Ginger & Lemon Tea	1 teabag	0.15	0.11±0.01	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Nerada Ginger & Lemon Tea	1 teabag	0.04	0.04±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

7.5 Discussion

In this study, the concentration of the principle gingerol and shogaol compounds was analyzed in 20 commercially available ginger products. The amount of each compound was relatively consistent across all products with [6]-gingerol and its degradation product, [6]-shogaol detected in the greatest concentrations and [8]-gingerol and [10]-shogaol in the smallest concentrations. The greater concentration of [6]-gingerol is to be expected as [6]-gingerol is the primary non-volatile pungent compound within the oleoresin of ginger.

This analysis also demonstrated a substantial variation in bioactive compounds between products. Per gram, ginger supplements, particularly the standardized extracts, contained the greatest concentration of measured compounds, while the concentration of compounds within other product categories varied considerably. These findings are similar to the results of previously published analyses by other groups.[12,13] The results of the current study expand on these previous studies by increasing the range of analyzed products and by including an additional compound, [10]-shogaol, in the analysis. [10]-shogaol has not been extensively studied; however, *in vitro* research suggests that it exerts anti-inflammatory properties and might aid wound healing[14,15] Furthermore, as part of the study protocol, the yield of the two extraction procedures used in this study was validated using a mix of ginger standards of a predetermined quantity. This processes improves the accuracy of our results and is a significant strength as this procedure has not been conducted consistently in previous studies.

Previous studies reported that the total concentration of active components within commercially available dietary supplements varied considerably from the manufacturers' claims.[16,17] In contrast, we found that the concentrations of gingerols and shogaols in three of the standardized extracts included in this analysis were consistent with those published by the manufacturers.

When analysed in terms of the approximate concentration that would be consumed in one recommended serve of each product, there were dietary supplements as well as some confectionary and beverage products that contained large concentrations of the analysed compounds. Ginger ales and confectionary are often recommended by health professionals to treat nausea. Although the smallest effective dose of ginger is yet to be elucidated, these results demonstrate that it is feasible to achieve an intake of the principle active compounds of ginger by consuming certain commercially available products that is comparable to the majority of dietary supplements analyzed in this study. For example, a large RCT (N=576) reported that two dosages of a standardized ginger extract, 0.5 and 1g, were effective in reducing chemotherapy-induced nausea and vomiting.[18] The effective daily dose of gingerol and shogaol for each dosage was 17mg and 34mg, respectively. In our analysis, four confectionary and beverage products contained a total gingerol and shogaol concentration similar to the dosage used by Ryan et al.[18] and the dietary supplements included in the current analysis. Therefore, these results also suggest that it is indeed feasible to attain equivalent amounts of gingerol and shogaols through dietary intake as indicated by in previous clinical trials.[18] This demonstrates the need for the consumption of ginger products to be monitored during future clinical trials

investigating ginger because the consumption of additional ginger products is likely to influence the effective total intake of ginger compounds. Furthermore, previous studies have demonstrated a potential ceiling effect with higher doses (2g) of ginger supplementation resulting in less or no control of symptoms compared to lower doses.[2] The consumption of additional ginger products could potentially increase the total consumption of active compounds in excess of a therapeutic dosage.

In addition, as previously noted by our group as well as other authors, the lack of analysis of ginger preparations and the lack of standardized extracts used in clinical trials could be responsible for the sometimes conflicting results reported due to the variability in active compounds.[2,13,19] The results of this study support this concern as there was a large difference in the detected concentration of each investigated product, including the ginger supplements. Hence, the measurement of the concentration of the active compounds within ginger products is indicated in future trials.

It should be noted that ginger contains a wide variety of bioactive compounds that have potentially beneficial properties.[20] Therefore, while this study was able to determine the concentration of an expanded range of these principle compounds compared to previous studies, other potentially important ginger compounds were not able to be analyzed due to the lack of commercial standards. Investigation of the concentrations of other compounds such as zingibain and dehydroshogaols in commercial products is recommended in future studies as these compounds have also demonstrated biological activity relevant to chronic conditions.[20] A second limitation of this study is that some of

the samples used were purchased commercially from nearby stores. The concentration of compounds could have been influenced by factors such as storage conditions; therefore, the results of this analysis might not be representative of the product when stored in different conditions. There is also the possibility that there was a variation in the concentration of analyzed compounds between different batches of the same product, particularly if different batches are sourced from multiple locations. This study analyzed a single sample of each product and so future trials may benefit from analyzing multiple batches of the same product.

7.6 Conclusion

Using RP-HPLC technology**,** an analysis of 20 commercially available ginger products demonstrated wide variation in the total amount of gingerols and shogaols within different ginger products, with standardized ginger extracts and spices containing the largest concentration of compounds. Biscuits and teas contained the least concentrations of the relevant compounds. When calculated by serving, certain beverages and confectionary contained similar or greater concentrations of the analysed compounds to the ginger supplements; hence future clinical trials investigating ginger formulations should ensure that the consumption of other ginger products is monitored so as to not confound the study results. In addition, the inconsistent concentration of active compounds within ginger supplements demonstrates the need for standardized extracts and the use of RP-HPLC in clinical trials to ensure sufficiently potent interventions.

7.7 Acknowledgments

We would like to thank Helsinn Integrative Care for supplying one of the ginger extracts for this study.

7.8 References

1. Bhattarai S, Tran VH, Duke CC. The stability of gingerol and shogaol in aqueous solutions. J Pharm Sci. 2001;90(10):1658-1664.

2. Marx WM, Teleni L, McCarthy AL, et al. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. Nutr. Rev. 2013;71(4):245-254.

3. Khandouzi N, Shidfar F, Rajab A, Rahideh T, Hosseini P, Mir Taheri M. The effects of ginger on fasting blood sugar, hemoglobin a1c, apolipoprotein B, apolipoprotein a-I and malondialdehyde in type 2 diabetic patients. Iran J Pharm Res. 2015;14(1):131-140.

4. Altman RD, Marcussen KC. Effects of a ginger extract on knee pain in patients with osteoarthritis. Arthritis Rheum. 2001;44(11):2531-2538.

5. Thomson M, Corbin R, Leung L. Effects of ginger for nausea and vomiting in early pregnancy: a meta-analysis. Journal of the American Board of Family Medicine : JABFM. 2014;27(1):115-122.

6. Viljoen E, Visser J, Koen N, Musekiwa A. A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting. Nutr J. 2014;13:20.

7. Marx W, Ried K, McCarthy AL, et al. Ginger-Mechanism of Action in Chemotherapy-induced Nausea and Vomiting: A Review. Crit Rev Food Sci Nutr. 2015:0.

8. Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. J Ethnopharmacol. 2010;127(2):515-520.

9. Molassiotis A, Brearley SG, Stamataki Z. Use of antiemetics in the management of chemotherapy-related nausea and vomiting in current UK practice. Support Care Cancer. 2011;19(7):949-956.

10. Forster D, Denning A, Wills G, Bolger M, McCarthy E. Herbal medicine use during pregnancy in a group of Australian women. BMC Pregnancy Childbirth. 2006;9:21.

11. Holst L, Wright D, Haavik S, Nordeng H. The use and the user of herbal remedies during pregnancy. J Altern Complement Med. 2009;15(7):787 - 792.

12. Schwertner H, Rios D, Pascoe J. Variation in concentration and labeling of ginger root dietary supplements. Obstet Gynecol. 2006;107:1337 - 1343.

13. Schwertner HA, Rios DC. High-performance liquid chromatographic analysis of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in ginger-containing dietary supplements, spices, teas, and beverages. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;856(1-2):41-47.

14. SYBYL-X 2.1 [computer program]. 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.: Tripos International.

15. van Breemen RB, Tao Y, Li W. Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale). Fitoterapia. 2011;82(1):38-43.

16. Chen C-Y, Cheng K-C, Chang AY, Lin Y-T, Hseu Y-C, Wang H-M. 10-Shogaol, an Antioxidant from Zingiber officinale for Skin Cell Proliferation and Migration Enhancer. International Journal of Molecular Sciences. 2012;13(2):1762-1777.

17. Harkey MR, Henderson GL, Gershwin ME, Stern JS, Hackman RM. Variability in commercial ginseng products: an analysis of 25 preparations. Am J Clin Nutr. 2001;73(6):1101-1106.

18. Heptinstall S, Awang DV, Dawson BA, Kindack D, Knight DW, May J. Parthenolide content and bioactivity of feverfew (Tanacetum parthenium (L.) Schultz-Bip.). Estimation of commercial and authenticated feverfew products. The Journal of pharmacy and pharmacology. 1992;44(5):391-395.

19. Ryan JL, Heckler CE, Roscoe JA, et al. Ginger (Zingiber officinale) reduces acute chemotherapy-induced nausea: a URCC CCOP study of 576 patients. Support Care Cancer. 2012;20(7):1479-1489.

20. Marx W, Kiss N, Isenring L. Is ginger beneficial for nausea and vomiting? An update of the literature. Current Opinion in Supportive and Palliative Care. 2015;9(2):189-195.

21. Chrubasik S, Pittler M, Roufogalis B. Zingiberis rhizoma: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine. 2005;12:684 - 701.

Chapter 8. Can ginger ameliorate chemotherapy-induced nausea? Protocol of a randomized double blind, placebo-controlled trial

Using the information gleaned from the systematic literature review in Chapter 2, this chapter details the protocol and methods of a randomized, double-blind, placebo-controlled clinical trial. This chapter was published in *BioMed Central Alternative and Complementary Medicine* (2014 Impact factor: 2.02) and received a 'Highly Accessed' classification due to the high volume of views (>1000) within the first months of publication. It had been cited three times (07/08/2015; Scopus) at the time of submission of this thesis.

Citation:

Marx W, McCarthy A, Ried K, Vitetta L, McKavanagh D, Thomson D, et al. Can ginger ameliorate chemotherapy-induced nausea? Protocol of a randomized double blind, placebo-controlled trial. *BMC Complementary and Alternative Medicine*. 2014;14(1):134. PubMed PMID: doi:10.1186/1472-6882-14-134.

8.1 Abstract

8.1.1 *Background*

Preliminary research shows ginger may be an effective adjuvant treatment for chemotherapy-induced nausea and vomiting but significant limitations need to be addressed before recommendations for clinical practice can be made.

8.1.2 *Methods/design*

In a double–blinded randomised-controlled trial, chemotherapy-naïve patients will be randomly allocated to receive either 1.2 g of a standardised ginger extract or placebo per day. The study medication will be administrated as an adjuvant treatment to standard anti-emetic therapy and will be divided into four capsules per day, to be consumed approximately every 4 hours (300 mg per capsule administered q.i.d) for five days during the first three cycles of chemotherapy. Acute, delayed, and anticipatory symptoms of nausea and vomiting will be assessed over this time frame using a valid and reliable questionnaire, with nausea symptoms being the primary outcome. Quality of life, nutritional status, adverse effects, patient adherence, cancer-related fatigue, and CINV-specific prognostic factors will also be assessed.

8.1.3 *Discussion*

Previous trials in this area have noted limitations. These include the inconsistent use of standardized ginger formulations and valid questionnaires, lack of control for anticipatory nausea and prognostic factors that may influence individual CINV response, and the use of suboptimal dosing regimens. This trial is the first to address these issues

by incorporating multiple unique additions to the study design including controlling for CINV-specific prognostic factors by recruiting only chemotherapy-naïve patients, implementing a dosing schedule consistent with the pharmacokinetics of oral ginger supplements, and independently analysing ginger supplements before and after recruitment to ensure potency. Our trial will also be the first to assess the effect of ginger supplementation on cancer-related fatigue and nutritional status. Chemotherapy-induced nausea and vomiting are distressing symptoms experienced by oncology patients; this trial will address the significant limitations within the current literature and in doing so, will investigate the effect of ginger supplementation as an adjuvant treatment in modulating nausea and vomiting symptoms.

8.1.4 *Trial registration*

ANZCTR.org.au Identifier: ACTRN12613000120774.

8.1.5 *Keywords*

Ginger, CINV, Nausea

8.2 Background

8.2.1 *Chemotherapy-induced nausea and vomiting places a significant burden on the patient*

Despite the efficacy of cytotoxic interventions in the treatment of cancer, these treatments are often accompanied by a variety of adverse effects. Chemotherapy-induced nausea and vomiting (CINV) is a relatively common side effect of this treatment and has been repeatedly rated as one of the most distressing symptoms in this setting [1,2]. While there have been multiple classes of medications developed to treat this symptom, nausea and vomiting persists in a large number of patients. The incidence of vomiting has been significantly reduced through combinations of anti-emetic medications, but efforts to control nausea have been less successful. Affecting upwards of 60% of patients [3], CINV has also been shown to significantly impact on patient quality of life (QoL). Moreover, although it happens rarely, CINV can be so severe that it can lead to dose reduction or treatment discontinuation, and subsequently increase the risk of disease progression [3-5].

8.2.2 *Ginger extract appears beneficial in treating chemotherapy-induced nausea and vomiting*

Ginger (*Zingiber officinale*) has been used for centuries by many cultures as a folk-remedy for gastrointestinal-related conditions [6]. Previous clinical trials have provided support for its use in the treatment of nausea in multiple settings including CINV [7-9] and two previous clinical trials have found ginger supplementation to be as effective as

metoclopramide in reducing symptoms of CINV [10,11]. Furthermore, animal and cell culture data have demonstrated a viable mechanism of action for its anti-nausea effect [12].

The rhizome of ginger possesses an array of bioactive compounds (i.e. gingerols, shogaols, zingiberene, zingerone, and paradol) that may be responsible for the reported beneficial effects of ginger use. Cell culture and animal studies suggest that these constituents stimulate oral and gastric secretions [13], regulate gastrointestinal motility [14,15], interact with the 5-HT_3 and NK-1 receptors implicated in the CINV reflex[16,17], and assist in rescuing intracellular redox metabolism [18]. Of note, the interaction of ginger with 5-HT_3 and NK-1 receptors is particularly promising as the success of modern anti-emetic medications (i.e. 5-HT_3 and NK-1 antagonists) are due to similar interactions with these same receptors. Furthermore, animal studies provide preliminary support for the role of ginger supplementation in the prevention of cisplatin-induced emesis [19,20].

A recent review found seven clinical studies have investigated ginger supplementation in this setting [21]. These studies present a contentious picture of the efficacy of ginger as an anti-CINV treatment in patients administered chemotherapy, with three demonstrating a positive effect, two in favour but with caveats, and two reporting no effect on measures of CINV. However, multiple limitations were identified within the existing literature that need to be resolved before clinical recommendations can be made.

8.2.3 *Chemotherapy-induced nausea and vomiting poses a significant risk to patients' nutritional status and treatment outcomes*

Previous studies report that approximately 50% of patients in the oncology setting are malnourished [22]. Malnutrition is a serious concern for oncology patients as it can significantly and severely affect QoL and treatment-related outcomes. Malnutrition can result in compromised immune function, reduced functional status, decreased performance status, and impaired treatment response [23-25]. Nausea and vomiting in this setting are of significant concern in patients diagnosed with cancer, as these symptoms can adversely affect dietary intake, increasing the risk of malnutrition during treatment.

It is feasible then to suggest that interventions that improve nausea and vomiting during chemotherapy may consequentially aid in improving and maintaining nutritional status. However, to date, there are no studies that have investigated the influence of ginger on patient nutritional status in this setting. Therefore, this protocol assesses nutritional status after each cycle using the validated questionnaire, the Patient Generated- Subjective Global Assessment, performed by an accredited dietitian.

8.2.4 *Chemotherapy-induced nausea and vomiting might exacerbate or be physiologically related to chemotherapy-related fatigue*

Like nausea and vomiting, chemotherapy-related fatigue (CRF) is both highly prevalent in this population and can significantly influence the patient's quality of life [26,27]. The

results from a number of studies that have investigated CRF have found nausea and vomiting to be a strongly associated set of symptoms [28]. The reason for this is not fully elucidated but due to this significant correlation, treatment options that have been traditionally targeted at treating CINV should be further investigated as these modalities may also provide benefit to patients experiencing CRF. Using the Functional Assessment of Chronic Illness Therapies- Fatigue (FACIT-F) subscale, our study will be the first to investigate the effect of adjuvant ginger supplementation on self-reported measures of fatigue.

8.2.5 *Comprehensive, validated questionnaires are required to assess chemotherapy-induced nausea and vomiting*

In order to assess CINV, the instrument used needs to be able to accurately capture the relevant aspects of CINV. Nausea, vomiting and retching, while temporally related, are distinct phenomena and therefore, are required to be measured as separate entities. In addition to this, a well-developed questionnaire should be able to provide a detailed picture of each phenomena. Widely used questionnaires in this setting include questions that measure multiple domains of CINV such as the severity, the perceived intensity of CINV; frequency, the amount of times CINV occurred over a time period; duration, the length of time that these symptoms persisted; and distress, the perceived burden that these symptoms place on the patients daily function and QoL [29].

There have been several questionnaires developed for the use of measuring nausea and vomiting, not only in the chemotherapy setting but also in other areas. A recent review identified 25 instruments that have been developed to measure nausea and vomiting in the clinical setting [29]. The authors used a list of criteria to determine the scope of nausea and vomiting that each questionnaire was able to capture. Of all questionnaires reviewed, no one tool fulfilled all criteria; however, the Index of Nausea, Vomiting, and Retching (INVR) tool was found to best meet this criteria [30].

A recent review found that only one previous study that investigated the effect of ginger on CINV used the INVR questionnaire [21]. This poses a significant limitation to the current literature as it is plausible that in these previous studies, ginger may have provided some benefit to domains of CINV that were not captured by the questionnaires employed in these respective studies.

Therefore, in order to ensure that our study is able to capture all relevant factors involved in CINV, it is important to use a questionnaire that is both validated and comprehensive and so it was decided that this study will incorporate the INVR questionnaire.

8.2.6 *Predisposing factors influence individual susceptibility to chemotherapy-induced nausea and vomiting*

Multiple factors are reported to influence the individual risk of a patient developing CINV [31]. These factors relate not only to the treatment protocol but also the

patient's lifestyle, mental state, and previous experience with nausea and vomiting in other settings [32-34]. Consequently, while the emetogenicity of the treatment protocol is the major determinant of CINV risk, a patient with multiple predisposing factors can experience significant levels of CINV despite being prescribed a low emetogenic chemotherapy regimen. Of particular concern is the development of anticipatory nausea and vomiting, a conditioned response that is difficult to treat, and the gradual resistance to anti-emetic therapy after multiple chemotherapy cycles [35].

These factors represent a significant set of potential confounding variables for RCTs in this setting. To date, all trials in this area have recruited patients that have already experienced nausea and vomiting in previous chemotherapy cycles. This allows for the potential recruitment of patients with an already established resistance to additional anti-emetic therapies. Furthermore, if lifestyle factors such as alcohol intake and previous experience of motion sickness, which have been shown to influence CINV risk, are not screened for, this may result in a study comprised of two groups with a predisposed heterogeneous response to CINV. To date, this has not been thoroughly controlled for and therefore, may account for some of the difference in the results between previous trials. We have developed a short questionnaire that aims to assess these factors and will be the first study to factor this into our post-study statistical analysis.

8.2.7 *Previous dosing regimens and formulations of ginger may not have been optimal*

In two recent studies that investigated the pharmacokinetics of multiple ginger compounds, it was found that these compounds have a relatively short half-life of approximately 1.5-3 hours [36,37]. In order to ensure that there are sufficient plasma levels of the active compounds throughout the day, the dosage in this study is divided between 4 capsules that will be consumed approximately every 4 hours.

The dosage of 1.2 g was selected for the following reasons: 1) it is within the typical dosage utilised in previous literature; 2) a lower dose, divided into multiple capsules, might not reach adequate concentrations to be effective; and 3) concerns that higher doses would reduce CINV control. Previous studies indicated higher doses were either less effective or possibly interfered with standard anti-emetic medications [38].

An additional limitation in the existing literature is the inconsistent use of standardized ginger extracts. Of the seven studies included in a recent review, only two studies used a ginger formulation that had been standardised to the relevant bioactive compounds while the remaining five used a crude ginger powder in capsule form [21]. The concentration of active compounds found within preparations of ginger has been found to be highly variable and can be influenced by the storage, location, and type of processing involved in the manufacturing of a specific ginger product [39]. Due to the majority of previous studies using unstandardized formulations, the inconsistent results

reported in the literature may be attributed to the differences in compounds the formulations used in each study.

To control for this limitation, we are using a ginger extract that has been standardised to contain 5% gingerols. We have also arranged for a sample of our ginger capsules to be independently analysed at the commencement and completion of our study to ensure the potency of the formulation.

Incorporating the results of these studies, we are expanding on the current literature as the majority of previous trials have used dosing regimens that are inconsistent with these findings.

8.3 Purpose of study and objectives

8.3.1 *Purpose of study*

Despite advances in anti-emetic medication, CINV continues to be a significant problem for many patients undergoing chemotherapy and is often rated as one of the most deleterious side-effects of cancer chemotherapeutic treatments. There is evidence from international trials that ginger formulations, in conjunction with standard anti-emetic medication, can be effective in the treatment of CINV. However, this therapy is not routinely used in oncology clinics due to its novelty and the lack of information about how patients will tolerate ginger in the clinical setting.

8.3.2 *Hypothesis*

It is hypothesised that in chemotherapy-naïve medical oncology patients about to commence treatment of any emetogenicity, adjuvant ginger supplementation compared with placebo will:

1. Reduce the frequency, distress and duration of chemotherapy-induced nausea (i.e. acute, delayed and anticipatory) during each chemotherapy cycle (up to 3 cycles).
2. Reduce frequency, distress and duration of chemotherapy-induced vomiting and retching
3. Result in improved nutritional status, physical function and quality of life
4. Be adhered to (>80% consumption of supplements) and well tolerated (no significant adverse events related to ginger supplementation).

8.3.3 *Outcomes*

8.3.3.1 Primary outcomes

- The frequency, severity, duration of acute and delayed nausea

8.3.3.2 Secondary outcomes

- The frequency and severity of acute and delayed vomiting
- The frequency and severity of acute and delayed retching
- Change in ratings of cancer-related fatigue
- Adequacy of supplement blinding

- Change in nutrition status
- Incidence and severity of symptoms associated with treatment
- Change in quality of life
- Change in quality of life caused by nausea and vomiting
- Patient adherence to intervention
- Influence of previously identified factors that affect the generation of CINV

8.4 Investigational plan

8.4.1 *Overall study design*

This study will be a double-blinded, randomised, placebo-controlled trial. Outcomes will be assessed at three days prior to chemotherapy, one day prior to chemotherapy, on the day of chemotherapy, and during the 4 days post-chemotherapy. Participants will consume the study medication for 5 days per chemotherapy cycle, commencing on the day of chemotherapy. This will be repeated over 3 chemotherapy cycles.

8.4.2 *Setting*

The trial will be initially conducted at the Princess Alexandra Hospital, Brisbane, Australia. Additional sites will be utilised if further funding is obtained.

8.4.3 *Eligibility criteria*

8.4.3.1 Inclusion criteria

The following inclusion criteria will apply:

- Chemotherapy-naive patients receiving chemotherapy of any emetogenicity level [40].
- >18 years old
- Life expectancy >3 months
- Baseline Karnofsky score >60
- No concurrent neoplasms or illness that induces nausea independent of chemotherapy
- No self-prescribed therapies or complimentary products used for nausea

8.4.3.2 Exclusion criteria

The following exclusion criteria will apply:

- Patients requiring radiotherapy
- Pregnant or lactating
- Concurrent use of other ginger-containing supplements and ingestion of large quantities of ginger
- History of adverse reactions to ginger

- Patients with malignancies of gastrointestinal tract / gastrointestinal diseases or nausea and vomiting due to reasons other than chemotherapy
- Thrombocytopenia or patients undergoing chemotherapy that, according to physician discretion, is likely to cause thrombocytopenia (platelets <50 × 10^9/L)
- Currently prescribed warfarin or on anti-coagulant therapy

8.5 Study treatment

8.5.1 *Ginger extract*

The experimental treatment will be a commercial ginger extract manufactured by Bluebonnet Nutrition [41]. This preparation is in capsule form, and is standardised to contain 5% gingerols. Each capsule contains 300 mg of ginger extract with 15 mg of active ingredient per capsule (60 mg per 1.2 g) within white gelatine capsules.

A regimen of 4 capsules per day will be selected in order to incorporate the pharmacokinetics of ginger [36,37].

8.5.2 *Placebo*

The placebo capsules will be identical to the ginger capsules in appearance and will contain 300 mg of an inert filler.

8.5.3 *Independent analysis*

The ginger capsules will be independently analysed for the active compounds (gingerols and shogaols) by the Southern Cross Plant Science Department at Southern Cross University using a standardised HPLC analysis method by the US Pharmacopeia (USP). Three random samples will be analysed at the beginning of the trial as well as at the end of the trial in order to assess the stability of the bioactive ingredients.

8.6 Concomitant treatment

All anti-emetic medication prescribed by the patient's medical team, including 5-HT3 antagonists (e.g. ondansetron), corticosteroids (e.g. dexamethasone), and NK1 receptor antagonists (e.g. aprepitant), will be permitted during this trial.

Participants will be advised to avoid consuming large amounts of dietary ginger or additional ginger capsules as well as any other adjuvant or alternative therapy for nausea and vomiting (excluding prescribed anti-emetic medication) during the study period.

Large amounts of ginger is defined as consumption of one serve of either ginger ale, crystallized ginger, or ginger containing meals/products most days (4/7) of the week for the past month; particularly within the week before and during chemotherapy.

8.7 Withdrawal criteria

Any participant who has been randomised and then withdraws will be included in the study on an intention to treat basis with patient consent. If a participant withdraws consent, data will be collected up until their time of withdrawal. Primary outcome data will be collected in these participants where possible.

Any participant who withdraws before being randomised (i.e. allocated to a particular study treatment) will be replaced, so that the next consenting participant receives the randomisation sequence and that participant's allocated study treatment.

8.8 Study duration

Participants will be enrolled in the study from the time of entry into the trial, through to 4 days after their third chemotherapy session. It is anticipated that it will take one year to recruit the necessary number of participants.

8.9 Treatment assignment and randomisation

Participant numbers will be assigned sequentially to participants as soon as they sign the informed consent form. Participants will be randomly assigned using a computer generated randomisation sequence. The randomisation sequence will be kept separately from the study investigators and will be generated by an independent researcher.

8.10 Methods

8.10.1 *Recruitment*

Participants will primarily be recruited through the daily chemotherapy education sessions that are offered by the hospital to patients who have been recently prescribed chemotherapy. Additionally, oncology nursing staff and chemotherapy-scheduling staff will be made aware of the study and will be encouraged to refer patients who may be interested in the study for further screening.

8.10.2 *Screening*

Patients will be assessed to ensure that they meet the inclusion and exclusion criteria. All patients who meet the criteria will be invited to participate in the study and be given a participant information sheet. This process may occur at any stage up to seven days prior to chemotherapy.

At the screening, patients will be informed that if they consume large amounts of dietary ginger or additional ginger capsules, as well as any other adjuvant or alternative therapy for nausea and vomiting (excluding prescribed anti-emetic medication), that this should be stopped at least 1 week prior to chemotherapy.

8.10.3 *Questionnaires used*

8.10.3.1 Rhodes Inventory of Nausea, Vomiting and Retching (INVR)

The INVR is a self-report questionnaire that measures nausea, vomiting and retching as separate entities based on 8 items with 5-point Likert scales [30]. The frequency and distress of all entities is measured as well as the duration of nausea and the amount of vomitus. The tool is suitable for use during each phase of CINV (i.e anticipatory, acute, and delayed) and is designed to measure symptoms over a 12 hour period; however, for the purpose of this trial, this period was extended to 24 hours to reduce the study burden on patients. It takes less than 5 minutes to complete.

8.10.3.2 The Functional Living Index – Emesis – 5 Day Recall. (FLIE-5DR)

The FLIE-5DR is a validated nausea and vomiting-specific self-reported outcome measure that investigates the specific impact of chemotherapy-related nausea and vomiting on patients' activities of daily living [42]. It has 9 items in each of the nausea and vomiting scales, the first item of which rates the extent of nausea or vomiting experienced in the previous 5 days. The remaining items examine patients' social, recreational and leisure activities, ability to do normal tasks, their enjoyment of eating and drinking, and the hardship caused by their nausea and vomiting on themselves and their carers. Each response is ranked on a seven point scale. The FLIE score is determined by summing the responses to the 9 questions in each scale. Therefore, the range of total scores possible per scale is 9 to 63, with a higher score responding to less hardship and

less impact of nausea or vomiting on daily life [42]. No or minimal impact on daily life is defined as an average FLIE item score of no more than 6 on the 7 point scale or a total FLIE score of more than 54 [42]. The FLIE has excellent internal reliability, with a Cronbach's $\alpha > 0.90$ for both sub-scales on all assessment points [43,44]. The FLIE takes less than 2 minutes to complete.

8.10.3.3 CINV susceptibility questionnaire

This questionnaire has been developed for use in this trial to determine participants' predisposition to CINV. Previous research has reported several factors correlated with susceptibility to CINV. These include lifestyle factors (e.g. alcohol intake); previous experience of nausea and/or vomiting from causes other than chemotherapy (e.g. motion sickness, pregnancy); and participant characteristics (e.g. age, gender). It is estimated that the questionnaire takes approximately 5 minutes to complete.

8.10.3.4 Edmonton Symptom Assessment System (ESAS)

The ESAS is a validated and reliable tool used to assess the severity of common symptoms experienced by cancer patients including pain, anxiety and drowsiness. It includes 10 items that are self-assessed by the patient using individual 10-point scales. This tool has been validated in this population and has reported a Cronbach's α of 0.79 [45]. The tool will be administrated at -1 day and at 4 days post-chemotherapy for each cycle in order to determine treatment related side-effects. The tool should take approximately 5 minutes to complete.

8.10.3.5 Patient Generated - Subjective Global Assessment (PG-SGA)

Nutritional status will be measured using the valid and reliable scored PG-SGA. [46]. Using the data gained from this tool, statistical analysis will be conducted to determine the impact of CINV on the participants' nutritional status. The PG-SGA will be conducted by a dietitian who has undergone training and testing for inter-rater reliability on nutritional status measures. The PG-SGA is specifically designed to assess the nutritional status of cancer patients. This tool provides a global rating of either A (well nourished), B (suspected or moderately malnourished) or C (severely malnourished). This global rating is based upon weight change, dietary intake, GI symptoms, a physical examination and the patient's functional capacity. A total PG-SGA score is also calculated. A higher score reflects a higher risk of malnutrition and an increased need for nutrition intervention and symptom management.

8.10.3.6 Functional Assessment of Cancer Therapy- General (FACT-G) and Fatigue (FACIT-F) subscale

The participants' self-assessed QoL will be measured using the FACT-G questionnaire, a validated tool that has been widely used in this setting [47]. It contains 27 questions with a 5-point scale, which assesses four domains of patient QoL: physical well-being, social/family well-being, emotional wellbeing, and functional well-being. Strong concurrent validity with the Functional Living Index-Cancer tool was demonstrated with a Pearson coefficient of 0.79 [47]. Additionally, we have included the

Functional Assessment of Chronic Illness Therapy- Fatigue (FACIT-F) subscale, a 13 item Likert scale, to assess self-reported symptoms of fatigue before and after each chemotherapy cycle. It is estimated to take between 5-10 minutes to complete.

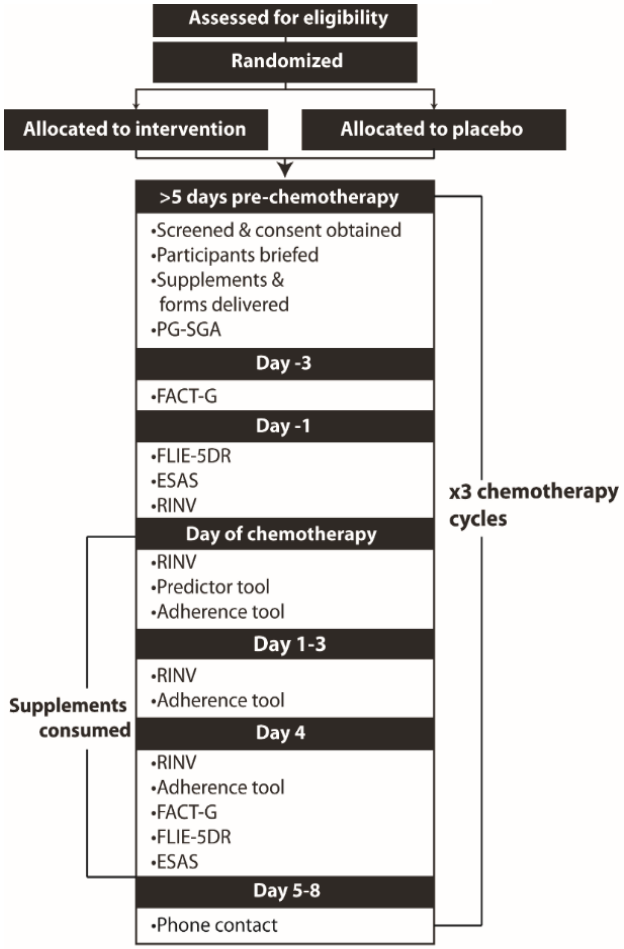
8.10.3.7 Adherence questionnaire

To assess the level of adherence to the study protocol, a questionnaire was developed for patients to record if and when they consumed the ginger/placebo doses per day. This is achieved by either recording the time or marking an X, depending on whether they consumed each dose, in the box corresponding to the dose in question. This is to be completed each day and is expected to take less than 2 minutes to complete.

8.11 Timeline

The details of the study procedure are detailed below in chronological order. This timeline contains the details of the study process per cycle and will be repeated for 3 cycles (Figure 8-1).

Figure 8-1 Study Flow Diagram



8.11.1 *Pre chemotherapy – 7 Days prior to chemotherapy*

The researcher will see patients as close as possible to 7 days prior to chemotherapy to determine their eligibility. If the patient is a viable candidate, informed consent will be obtained, the details of the study will be explained, and the supplements and questionnaire booklet will be delivered. Participants will be provided with written information and educated regarding the consumption of the supplement.

Participants will be randomised and provided with a 5 day (4 × 300 mg capsules per day) supply of ginger extract (20 capsules) or the placebo control (20 capsules) to be consumed daily with liquid in addition to their usual diet for 4 days, starting on the day of chemotherapy. The supplement will be provided in a sealed plastic container that will be packed by a researcher not involved in data collection.

Participants will be given a booklet containing the self-report questionnaires for CINV, QoL, adverse events, and blinding for the full study period per chemotherapy cycle (5 days).

Each booklet will contain:

- One INVR questionnaire per day: one on the day of chemotherapy, and one each of the 4 days post-chemotherapy.
- Two FACT-G/FACIT-F questionnaires per cycle.
- One FLIE-5DR questionnaire per cycle.
- Two ESAS questionnaire.
- One CINV susceptibility questionnaire.

- One Adherence Questionnaire for each day the participant receives the study medication: one on the day of chemotherapy, and one for each of the 4 days post-chemotherapy.
- Instructions on how and when to complete these questionnaires will be included, as well the contact details of the study investigators.

During this consultation, the participant's nutritional status will also be assessed using the PG-SGA assessment tool.

8.11.2 *Pre chemotherapy – 1 day prior to chemotherapy*

The following tools will be completed by the participant 24 hours before chemotherapy:

- One FACT-G/FACIT-F questionnaire
- One ESAS questionnaire

8.11.3 *Day of chemotherapy*

The following tools will be completed by the participant on the day of chemotherapy:

- One INVR questionnaire will be completed before chemotherapy commences.
- One CINV susceptibility questionnaire will be completed any time after chemotherapy has commenced.
- One adherence questionnaire.

Additionally, the participant is to consume one ginger/placebo capsule 1 hour before the administration of chemotherapy and then once every 4 hours after that for the remaining 3 capsules. The timing will be discussed with the participant to help ensure the participant understands the regimen.

8.11.4 *Post-chemotherapy – Day 1-4 Post-chemotherapy*

During the 4 days post-chemotherapy, participants will be asked to complete:

- One INVR questionnaire per day. The timing of completion should be at the same time of day as when they completed their previous questionnaire. This will ensure that 24 hours is assessed per questionnaire.
- One adherence questionnaire.
- The participant will consume 4 capsules per day. One before breakfast, one before lunch, one during an afternoon snack, and one before dinner. These capsules are to be consumed one hour before each meal.

8.11.5 *Post-chemotherapy – Day 4 Post-chemotherapy only*

At the end of day 4, participants will be asked to complete:

- One FACT-G/FACIT-F questionnaire
- One FLIE-5DR questionnaire
- One ESAS questionnaire
- One adherence questionnaire

Supplements are consumed using the same schedule as above.

At the end of day 4, participants will no longer be required to consume the oral supplements and any unconsumed supplements, along with the questionnaire booklet, will either be collected by the research team, along with the participant questionnaire booklet, during the participant's next visit to the hospital or sent directly to the researchers using a reply-paid envelope. Unconsumed supplements of each individual will be counted in order to determine their level of adherence to the study protocol.

8.12 Assessment of blinding

At the end of day 4, the investigator will contact each participant to obtain information regarding the study blinding. This will be determined by asking each participant the following questions: "Do you think you received the placebo or the ginger supplement and why do you think this?". Participants will also be asked if they have any comments or queries regarding the trial so as to gather feedback for the improvement of the study protocol for future trials.

The timing of the participant's next chemotherapy cycle will also be discussed and arrangements will be made to meet within the week before chemotherapy in order to dispense additional supplements and assessments.

8.13 Statistical analysis

Analyses will be conducted according to intention-to-treat principles i.e. the consent process will maximise outcome data collection and attempt to assess nausea

symptoms for everyone, and will retain original group allocation despite actual compliance levels.

Participants will be block stratified by chemotherapy category (i.e. minimal, low, moderate and high emetogenicity) then randomised within strata into intervention and control groups (Figure 8-1) [48].

Descriptive statistics will be presented as mean ± standard deviation, or median with range, as appropriate. Parametric analyses will be used for all continuous variables. Chi-square analyses will determine associations between categorical variables. For example, the incidence, severity and type of nausea and vomiting between the two groups. Pearson correlation analysis of continuous variables will be performed. Repeated measures analyses will be conducted to detect between group differences over time as per our statistician recommendations. Statistical significance will be set at $p < 0.05$ level (two-tailed). Data will be analysed using SPSS for Windows version 22 (Statistical Package for Social Sciences, IL, USA).

8.14 Sample size

A sample size calculation for comparing two means with unpaired t-tests based on the reductions in the prevalence of chemotherapy-induced nausea reported by Panahi et al. [49], estimates that 73 participants would be required in the intervention and control groups (i.e. total of N = 146) to detect this difference with 80 per cent power at the 95 per cent significance level (two tailed).

Approximately 250 patients receive moderately emetogenic chemotherapy and 240 patients receive highly emetogenic chemotherapy at Princess Alexandra Hospital each six months (1/3-1/9/2012) which indicates that the required sample size is obtainable in this study.

8.15 Ethical considerations

The study protocol has been approved by the Metro South Human Research Ethics Committee on the 4th of July, 2013. The trial has also been registered with the Australian New Zealand Clinical Trials Registry and has been assigned the identifier, ACTRN12613000120774. The study complies with the Declaration of Helsinki rules and the principles of Good Clinical Practice guidelines. Informed consent will be gained from all participants before commencing the trial and patient data will be stored securely. Participants will also be monitored for adverse effects and will be discontinued immediately if the study protocol is determined to be causing harm or if the participant chooses to withdraw. This study received grant funding from the Queensland Health – Health Practitioner Scheme.

8.16 Discussion

This study protocol expands on the current literature regarding the efficacy of ginger as an adjuvant therapy for CINV. Recommendations for the use of ginger in the oncology setting are premature, as previous reviews have shown inconsistent results and have possessed several limitations. Primary concerns identified in the literature include the lack of control of anticipatory nausea, the inconsistent use of standardised ginger

extracts and validated assessments tools, and a lack of assessment for prognostic factors that may influence individual CINV response [21]. Additionally, recent pharmacokinetic studies demonstrate that the half-life of the active compounds within ginger are relatively short-lived, which suggests that the dosing regimens employed by previous studies may be suboptimal. Furthermore, multiple studies included in these reviews have used anti-emetic therapies that are not congruent with current best practice and anti-emetic guidelines and therefore, the application of these previous findings to current practise are further diminished [21,48].

In designing our trial, we aimed to address these limitations while incorporating elements of rigorous study methodology that have been incorporated in previous trials in this area. For example, our trial will be using multiple, validated assessment tools along with a standardised ginger extract, both of which have been utilised in at least two previous trials [50,51]. We will, however, expand on this by independently analysing our extracts at both the beginning and end of our recruitment phase to ensure consistent potency.

It should also be noted that one study by Ryan et al. [51] found ginger to reduce CINV when ginger supplementation was commenced three days before chemotherapy. We, however, decided against using this methodology and opted for ginger supplementation commencing on the day of chemotherapy due to the following reasons. Firstly, there have been multiple previous trials using ginger for CINV as well as other forms of nausea that did not use the regimen used by Ryan et al. [51] but rather a

timeframe and dosage more closely resembling the regimen in our protocol that yielded beneficial results [49,52-54]. In addition to this, to date, there has been no research that has investigated the regimen used in the Ryan et al. [51] study compared to the more typical dosing regimen that has been employed in our study, which restricts one from determining the superiority of said regimen. Lastly, the basis for said regimen, from our research and from the discussion in the Ryan et al. [51] paper, seems to have been implemented largely on a theoretical basis and therefore, until more evidence arises, we have decided to instead opt for the more patient-convenient regimen described in this manuscript.

Our trial will also be the first to introduce multiple novel study design elements. Primarily, our study will be the first to recruit only chemotherapy-naive patients. This strategy should mitigate the significant limitation of anticipatory nausea. It is a response to previous research reporting that CINV control progressively deteriorates with each subsequent chemotherapy cycle, if not adequately controlled during the initial cycle [35]. Due to the association between fatigue and nausea in this setting, we will also investigate the effect that ginger has on this association in order to determine if ginger may be of benefit to patients also experiencing cancer-related fatigue. Finally, our study will also implement a dosing regimen that is consistent with the findings of the previously mentioned pharmacokinetic studies that will likely improve the CINV protection of this therapy. If successful, this trial will provide support for the efficacy of ginger as a viable

adjuvant anti-emetic therapy and in doing so, help manage chemotherapy symptoms and assist in improving patient QoL.

8.17 Abbreviations

CINV, Chemotherapy-induced Nausea and vomiting; QoL, Quality of life; PG-SGA, Patient generated - subjective global assessment; FACT-G, Functional assessment of cancer therapy- general; FACIT-F, Functional assessment of chronic illness therapy-fatigue; INVR, Rhodes inventory of Nausea vomiting and retching; ESAS, Edmonton Symptom Assessment System; FLIE-5DR, The functional living Index – Emesis – 5 day recall

8.18 Competing interests

Luis Vitetta has received National Institute of Complementary Medicine and National Health and Medical Research Council of Australia competitive funding and Industry support for research into nutraceuticals. No other author has any competing interests to disclose.

8.19 Authors' contributions

WM was responsible for the development of the manuscript, study design, and ethics submission, EI is the principle investigator, provided PhD supervision, management of funding, hiring of research assistant, and overall supervision of project progression, LV participated in manuscript development, product acquisition, provided PhD supervision, and information regarding the mechanism of action of ginger. KR provided PhD supervision and participated in the development of the study design and

manuscript development, ALM participated in the development of the study design, provided information regarding prognostic factors, and contributed to the development of the protocol manuscript. DT contributed with study design and development of manuscript as well providing background on emetogenic chemotherapy regimens. AS provided product knowledge, PhD supervision and participated in the design of the study. DM provided information regarding the safety of ginger supplementation, contributed to the development of the protocol manuscript with the manuscript development as well as the procurement of placebo capsules. All authors read and approved the final manuscript.

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8.21 References

1. Sun CC, Bodurka DC, Weaver CB, Rasu R, Wolf JK, Bevers MW, Smith JA, Wharton JT, Rubenstein EB: Rankings and symptom assessments of side effects from chemotherapy: insights from experienced patients with ovarian cancer. *Support Care Cancer* 2005, 13:219–227.

2. Carelle N, Piotto E, Bellanger A, Germanaud J, Thuillier A, Khayat D: Changing patient perceptions of the side effects of cancer chemotherapy. *Cancer* 2002, 95:155–163.

3. Bloechl-Daum B, Deuson RR, Mavros P, Hansen M, Herrstedt J: Delayed nausea and vomiting continue to reduce patients' quality of life after highly and moderately emetogenic chemotherapy despite antiemetic treatment. *J Clin Oncol* 2006, 24:4472–4478.

4. Vidall C, Dielenseger P, Farrell C, Lennan E, Muxagata P, Fernandez-Ortega P, Paradies K: Evidence-based management of chemotherapy-induced nausea and vomiting: a position statement from a European cancer nursing forum. *Ecancermedicalsci* 2011, 5:211.

5. Ballatori E, Roila F, Ruggeri B, Betti M, Sarti S, Soru G, Cruciani G, Di Maio M, Andrea B, Deuson RR: The impact of chemotherapy-induced nausea and vomiting on health-related quality of life. *Support Care Cancer* 2007, 15:179–185.

6. Baliga MS, Haniadka R, Pereira MM, D'Souza JJ, Pallaty PL, Bhat HP, Popuri S: Update on the chemopreventive effects of ginger and its phytochemicals. *Crit Rev Food Sci Nutr* 2011, 51:499–523.

7. Holtmann S, Clarke AH, Scherer H, Hohn M: The anti-motion sickness mechanism of ginger. A comparative study with placebo and dimenhydrinate. *Acta Otolaryngol* 1989, 108:168–174.

8. Willetts KE, Ekangaki A, Eden JA: Effect of a ginger extract on pregnancy-induced nausea: a randomised controlled trial. *Aust N Z J Obstet Gynaecol* 2003, 43:139–144.

9. Ernst E, Pittler MH: Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials. *Br J Anaesth* 2000, 84:367–371.

10. Manusirivithaya S, Sripramote M, Tangjitgamol S, Sheanakul C, Leelahakorn S, Thavaramara T, Tangcharoenpanich K: Antiemetic effect of ginger in gynecologic oncology patients receiving cisplatin. *Int J Gynecol Cancer* 2004, 14:1063–1069.

11. Sontakke S, Thawani V, Naik MS: Ginger as an antiemetic in nausea and vomiting induced by chemotherapy: a randomized, cross-over, double blind study. *Indian J Pharmacol* 2003, 35:5.

12. Chrubasik S, Pittler MH, Roufogalis BD: Zingiberis rhizoma: a comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine* 2005, 12:684–701.

13. Platel K, Srinivasan K: Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. *Int J Food Sci Nutr* 1996, 47:55–59.

14. Yamahara J, Huang QR, Li YH, Xu L, Fujimura H: Gastrointestinal motility enhancing effect of ginger and its active constituents. *Chem & Pharmaceutical bulletin* 1990, 38:430–431.

15. Wu KL, Rayner CK, Chuah SK, Changchien CS, Lu SN, Chiu YC, Chiu KW, Lee CM: Effects of ginger on gastric emptying and motility in healthy humans. *Eur J Gastroenterol Hepatol* 2008, 20:436–440.

16. Riyazi A, Hensel A, Bauer K, Geissler N, Schaaf S, Verspohl EJ: The effect of the volatile oil from ginger rhizomes (Zingiber officinale), its fractions and isolated compounds on the 5-HT3 receptor complex and the serotoninergic system of the rat ileum. *Planta Med* 2007, 73:355–362.

17. Abdel-Aziz H, Windeck T, Ploch M, Verspohl EJ: Mode of action of gingerols and shogaols on 5-HT3 receptors: binding studies, cation uptake by the receptor

channel and contraction of isolated guinea-pig ileum. *Eur J Pharmacol* 2006, 530:136–143.

18. Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN: Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. *J Ethnopharmacol* 2010, 127:515–520.

19. Sharma SS, Kochupillai V, Gupta SK, Seth SD, Gupta YK: Antiemetic efficacy of ginger (Zingiber officinale) against cisplatin-induced emesis in dogs. *J Ethnopharmacol* 1997, 57:93–96.

20. Sharma SS, Gupta YK: Reversal of cisplatin-induced delay in gastric emptying in rats by ginger (Zingiber officinale). *J Ethnopharmacol* 1998, 62:49–55.

21. Marx WM, Teleni L, McCarthy AL, Vitetta L, McKavanagh D, Thomson D, Isenring E: Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. *Nutr Rev* 2013, 71:245–254.

22. Isenring E, Cross G, Kellett E, Koczwara B, Daniels L: Nutritional status and information needs of medical oncology patients receiving treatment at an Australian public hospital. *Nutr Cancer* 2010, 62:220–228.

23. Davidson W, Teleni L, Muller J, Ferguson M, McCarthy AL, Vick J, Isenring EA: Malnutrition and chemotherapy-induced nausea and vomiting : implications for practice. *Oncol Nurs Forum* 2012, 39:E340-345.

24. Van Cutsem E, Arends J: The causes and consequences of cancer-associated malnutrition. *European J of Oncol nursing : the official J of European Oncol Nursing Soc* 2005, 9:S51–S63.

25. Tong HT, Isenring EA, Yates P: The prevalence of nutrition impact symptoms and their relationship to quality of life and clinical outcomes in medical oncology patients. *Support Care Cancer* 2008, 17:83–90.

26. Annunziata MA, Muzzatti B, Mella S, Bidoli E: Fatigue, quality of life, and mood states during chemotherapy in Italian cancer patients. *Tumori* 2013, 99:e28–33.

27. Irvine D, Vincent L, Graydon JE, Bubela N, Thompson L: The prevalence and correlates of fatigue in patients receiving treatment with chemotherapy and radiotherapy. A comparison with the fatigue experienced by healthy individuals. *Cancer Nurs* 1994, 17:367–378.

28. Oh HS, Seo WS: Systematic review and meta-analysis of the correlates of cancer-related fatigue. *Worldviews Evid Based Nurs* 2011, 8:191–201.

29. Wood JM, Chapman K, Eilers J: Tools for assessing nausea, vomiting, and retching. *Cancer Nurs* 2011, 34:E14–24.

30. Rhodes VA, McDaniel RW: The index of nausea, vomiting, and retching: a new format of the lndex of nausea and vomiting. *Oncol Nurs Forum* 1999, 26:889–894.

31. Rubenstein EB: The Role of Prognostic Factors in Chemotherapy-Induced Nausea and Vomiting. In *Management of Nausea and Vomiting in Cancer and Cancer Treatment.* Edited by Hesketh PJ. Sudbury, MA: Jones and Bartlett; 2005:87–98.

32. Booth CM, Clemons M, Dranitsaris G, Joy A, Young S, Callaghan W, Trudeau M, Petrella T: Chemotherapy-induced nausea and vomiting in breast cancer patients: a prospective observational study. *J Support Oncol* 2007, 5:374–380.

33. Roila F, Boschetti E, Tonato M, Basurto C, Bracarda S, Picciafuoco M, Patoia L, Santi E, Penza O, Ballatori E: Predictive factors of delayed emesis in cisplatin-treated patients and antiemetic activity and tolerability of metoclopramide or dexamethasone. A randomized single-blind study. *Am J Of Clin Oncol* 1991, 14:238–242.

34. Roscoe JA, Morrow GR, Colagiuri B, Heckler CE, Pudlo BD, Colman L, Hoelzer K, Jacobs A: Insight in the prediction of chemotherapy-induced nausea. *Support Care Cancer* 2010, 18:869–876.

35. Morrow GR, Roscoe JA, Hickok JT, Stern RM, Pierce HI, King DB, Banerjee TK, Weiden P: Initial control of chemotherapy-induced nausea and vomiting in patient quality of life. *Oncology (Williston Park)* 1998, 12:32–37.

36. Yu Y, Zick S, Li X, Zou P, Wright B, Sun D: Examination of the pharmacokinetics of active ingredients of ginger in humans. *AAPS J* 2011, 13:417–426.

37. Zick SM, Djuric Z, Ruffin MT, Litzinger AJ, Normolle DP, Alrawi S, Feng MR, Brenner DE: Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev* 2008, 17:1930–1936.

38. Zick SM, Turgeon DK, Vareed SK, Ruffin MT, Litzinger AJ, Wright BD, Alrawi S, Normolle DP, Djuric Z, Brenner DE: Phase II study of the effects of ginger root extract on eicosanoids in colon mucosa in people at normal risk for colorectal cancer. *Cancer Prev Res (Phila)* 2011, 4:1929–1937.

39. Schwertner HA, Rios DC, Pascoe JE: Variation in concentration and labeling of ginger root dietary supplements. *Obstet Gynecol* 2006, 107:1337–1343.

40. Hesketh PJ: Chemotherapy-induced nausea and vomiting. *N Engl J Med* 2008, 358:2482–2494.

41. BlueBonnet Nutrition. http://www.bluebonnetnutrition.com/.

42. Lindley CM, Hirsch JD, O'Neill CV, Transau MC, Gilbert CS, Osterhaus JT: Quality of life consequences of chemotherapy-induced emesis. *Qual Life Res* 1992, 1:331-340.

43. Brearly SG, Clements CV, Molassiotis A: A review of patients self-report tools for chemotherapy-induced nausea and vomiting. *Support Care Cancer* 2008, 16:1213–1229.

44. Cohen L, de Moor C, Eisenberg P, Ming E, Hu H: Chemotherapy-induced nausea and vomiting—incidence and impact on patient quality of life at community oncology settings. *Support Care Cancer* 2007, 15:497–503.

45. Chang VT, Hwang SS, Feuerman M: Validation of the edmonton symptom assessment scale. *Cancer* 2000, 88:2164–2171.

46. Bauer J, Capra S, Ferguson M: Use of the scored Patient-Generated Subjective Global Assessment (PG-SGA) as a nutrition assessment tool in patients with cancer. *Eur J Clin Nutr* 2002, 56:779–785.

47. Cella D, Hahn EA, Dineen K: Meaningful change in cancer-specific quality of life scores: differences between improvement and worsening. *Qual Life Res* 2002, 11:207–221.

48. Roila F, Herrstedt J, Aapro M, Gralla RJ, Einhorn LH, Ballatori E, Bria E, Clark-Snow RA, Espersen BT, Feyer P, *et al*: Guideline update for MASCC and ESMO in the prevention of chemotherapy- and radiotherapy-induced nausea and vomiting: results of the Perugia consensus conference. *Ann Oncol* 2010, 21:v232–v243.

49. Panahi Y, Saadat A, Sahebkar A, Hashemian F, Taghikhani M, Abolhasani E: *Effect of Ginger on Acute and Delayed Chemotherapy-Induced Nausea and Vomiting: A Pilot.* Open-Label Clinical Trial. Integr Cancer Ther: Randomized; 2012.

50. Zick S, Ruffin M, Lee J, Normolle D, Siden R, Alrawi S, Brenner D: Phase II trial of encapsulated ginger as a treatment for chemotherapy-induced nausea and vomiting. *Support Care Cancer* 2009, 17:563–572.

51. Ryan JL, Heckler CE, Roscoe JA, Dakhil SR, Kirshner J, Flynn PJ, Hickok JT, Morrow GR: Ginger (Zingiber officinale) reduces acute chemotherapy-induced nausea: a URCC CCOP study of 576 patients. *Support Care Cancer* 2012 , 20:1479-1489.

52. Pillai AK, Sharma KK, Gupta YK, Bakhshi S: Anti-emetic effect of ginger powder versus placebo as an add-on therapy in children and young adults receiving high emetogenic chemotherapy. *Pediatr Blood Cancer* 2011, 56:234–238.

53. Arfeen Z, Owen H, Plummer JL, Ilsley AH, Sorby-Adams RA, Doecke CJ: A double-blind randomized controlled trial of ginger for the prevention of postoperative nausea and vomiting. *Anaesthesia and Intensive care* 1995, 23:449–452.

54. Lien HC, Sun WM, Chen YH, Kim H, Hasler W, Owyang C: Effects of ginger on motion sickness and gastric slow-wave dysrhythmias induced by circular vection. *Am J Physiol Gastrointest Liver Physiol* 2003, 284:G481–489.

Chapter 9. The effect of a standardized ginger extract on chemotherapy-induced nausea and vomiting related quality of life in patients undergoing moderately and highly emetogenic chemotherapy: a randomized controlled trial.

Chapter 9 presents the results of the main study of this thesis, a double-blind, randomized, placebo-controlled trial that investigated the use of adjuvant ginger supplementation for chemotherapy-induced nausea and vomiting. This chapter discusses the strengths and limitations of this study and provides recommendations for clinical practice and future studies. Supplementary information to this trial can be found in the appendices.

The study protocol of this clinical trial was adapted from the protocol manuscript in Chapter 8. The only deviations are listed below:

- The primary outcomes were changed from the frequency, severity, duration of acute and delayed nausea to chemotherapy-induced nausea-related quality of life (QoL). The most consistent effect of nausea that is reported in the literature is the significant decline in QoL.[4,16,17] Therefore, in order to assess the effect of ginger supplementation on clinically-relevant outcomes, the primary outcome was changed in order to ensure the study was adequately powered to detect a

significant difference in the effect that CINV had on the participant's daily living and wellbeing.

- In order to investigate the feasibility of the published study protocol, a pilot study was undertaken at Princess Alexandra Hospital with 10 chemotherapy-naïve participants during their first cycle of chemotherapy. From this study, several parts of the study protocol were modified to improve the readability and participant acceptance of the questionnaires, the acceptability of the study capsules, and to streamline the recruitment process. In particular, a multidisciplinary approach was found to be the optimal strategy to identify potentially eligible patients. The final recruitment process involved the coordination with the chemotherapy nurses, haematology physicians, oncologists, dietitians, pharmacists, and cancer-care coordinators, who all provided assistance with this study. In addition, through this process, the hospital's daily chemotherapy education sessions were identified as a regular avenue to introduce eligible patients to the study. In these sessions, this PhD candidate would provide a short, informal presentation about the study and then arrange to speak with interested patients after the education session. Furthermore, participant feedback regarding the questionnaire booklet resulted in several minor revisions that improved its readability.
- Finally, the sample size was recalculated based on the results of the pilot study (N=10) and was revised to 77 participants. The previous sample size was calculated based on a previous study using the original primary outcome and a different questionnaire to that used in this study to capture the revised outcome.

9.1 Abstract

Ginger supplementation could be an effective adjuvant treatment for chemotherapy-induced nausea and vomiting (CINV); however, previous trials in this area have significant methodological limitations that preclude recommending the routine use of ginger in clinical practice.

The aim of this double-blind randomised controlled trial was to overcome these limitations and thereby determine the effect of adjuvant time- and dose-standardised ginger on chemotherapy-induced nausea (CIN)-related quality of life (QoL), compared to placebo, in chemotherapy-naïve patients over three cycles of moderately- and highly-emetogenic chemotherapy.

Fifty-one patients were randomly allocated to receive either 1.2 g of a standardised ginger extract or placebo per day, in addition to standard anti-emetic therapy. The supplements were divided into four capsules per day, consumed every four hours for five days during the first three cycles of chemotherapy. The primary outcome was CIN-related QoL measured with the Functional Living Index- Emesis (FLIE) questionnaire. Secondary outcomes included acute and delayed nausea, vomiting, and retching as well as cancer-related fatigue, nutrition status, and CINV-specific prognostic factors.

Over three consecutive chemotherapy cycles, nausea was more prevalent than vomiting (47% vs 12% of all participants experienced symptoms, respectively). In cycle 1, intervention participants reported significantly higher ratings of CIN-related QoL (Median [25^{th}, 75^{th} percentile] = 61.5 [56.1, 63] vs 54 [46, 63]; p=0.029), CINV-related QoL (Median [25^{th}, 75^{th} percentile] = 124.5 [113, 126] vs 111 [99.2, 126]; p=

0.043) and global QoL (Mean±standard deviation = 85.1±18.9 vs 71.9±18.3; p= 0.003) and less fatigue (Mean±standard deviation = 41.8±13.1 vs 32.2±10.8; p=0.007) than placebo participants. There were no significant results in cycle 2. In cycle 3, global QoL (Median [25^{th}, 75^{th} percentile] = 83.6±15.0 vs 75.1±13.9; p=0.040) and fatigue (Mean±standard deviation = 42.4±10.2 vs 36.1±7.2; p=0.013) were significantly better in the intervention group compared to placebo. There was no difference in reported adverse effects.

This trial suggests adjuvant ginger supplementation is associated with better chemotherapy-induced nausea-related quality of life and less cancer-related fatigue, with no difference in adverse effects compared to placebo.

9.2 Introduction

The prevention and management of chemotherapy-induced nausea and vomiting (CINV) is a priority in the oncology setting. While the development of a range of anti-emetic medications has considerably reduced the prevalence of CINV, vomiting and, in particular, nausea, are still experienced by up to 25% and 61% of cancer patients, respectively.[1] CINV is also associated with poor quality of life (QoL), malnutrition, and if persistent, can result in cancer treatment delays and dose reductions, culminating in poorer treatment outcomes.[2-4] Furthermore, when nausea and vomiting are measured separately, nausea is reported to affect QoL to a greater extent than vomiting. This suggests that additional interventions to control nausea are required.[3]

Various interventions to reduce CINV have been investigated. These include pharmaceuticals (e.g. olanzapine), behavioural interventions (e.g. progressive muscle relaxation), and nutraceuticals including ginger supplementation.[5-7] The compounds within ginger are understood to possess multiple properties relevant to the management of CINV, including 5-HT_3 receptor antagonism, which is one of the cornerstones of modern antiemetic drug therapies such as ondansetron and granisetron.[8]

Ginger has been trialled with some success for other types of nausea, including morning sickness and post-operative nausea and vomiting.[9-11] There is also mounting evidence supporting the use of adjuvant ginger to reduce CINV.[7] However, as discussed in our previous articles,[7,12] extant research has multiple methodological limitations that must be addressed in studies of ginger before this intervention can be

recommended as a complement to routine clinical practice. These limitations include the lack of control for prognostic factors, potentially suboptimal dosing regimens, and inconsistent use of validated questionnaires and standardized ginger products.[7,12] This study was designed to overcome these limitations.

The primary aim of this double-blind randomised controlled trial was to determine the effect, from baseline, of adjuvant time- and dose-standardised ginger on chemotherapy-induced nausea (CIN)-related QoL, compared to placebo, in chemotherapy naïve patients over three cycles of moderately- and highly-emetogenic chemotherapy.

Previously unexplored, but clinically important outcomes, were also investigated. Cancer-related fatigue and malnutrition are both prevalent in chemotherapy cohorts, are consistently associated with CINV, and are associated with significant decrements in patient QoL.[2,13] Hence, fatigue and malnutrition were also investigated in this trial to determine if adjuvant ginger supplementation could benefit these outcomes. In addition, the potential correlation between ginger and the anti-emetic medication aprepitant was assessed. This was prompted by the findings by Zick et al.[14], who reported worse control of delayed CINV in patients receiving 2g of ginger and aprepitant.

9.3 Methods

The design of this double-blind, randomised placebo-controlled trial is fully detailed in our published protocol manuscript.[12] The study protocol was approved by the Metro South Human Research Ethics Committee, Brisbane, Australia and was registered with the Australian New Zealand Clinical Trials Registry

(ACTRN12613000120774). The CONSORT checklist for randomised controlled trials was used to prepare this manuscript.[18]

9.3.1 *Sample and recruitment*

Patients were recruited if they were chemotherapy-naïve, were due to receive a moderately- or highly-emetogenic chemotherapy regimen, were at least 18 years old, had a baseline Karnofsky score >60,[15] had no known concurrent neoplasms or illness that induces nausea independent of chemotherapy, and did not self-prescribe therapies or complementary products used for nausea. Patients were excluded if they were scheduled to receive radiotherapy during the study period, were pregnant or lactating, concurrently used other ginger-containing supplements or ingested large quantities of ginger, had a history of adverse reactions to ginger, and thrombocytopenia. These inclusion and exclusion criteria were applied equally to both the intervention and placebo groups. Chemotherapy regimens were categorized as highly- or moderately-emetogenic consistent with the Multinational Association for Supportive Care and Cancer anti-emetic guidelines.[16] Written informed consent was obtained at time of enrolment.

Patients were recruited from the Princess Alexandra Hospital, Brisbane, Australia from March 2014 to February 2015. Potentially eligible patients were identified by research staff during daily chemotherapy education sessions and through the hospital chemotherapy scheduling system within one week prior to the first cycle of chemotherapy. Eligible patients were randomly allocated to ginger or placebo capsules, and received three questionnaire booklets, one for each cycle, which were

either mailed back to the researchers upon completion or collected during the following cycle.

9.3.2 *Intervention*

Participants were randomly assigned to receive either 1.2g (4 x 300mg) of a standardized ginger extract or placebo in conjunction with the standard antiemetic therapy prescribed by their physician. The ginger extract was standardised to contain 5% gingerols in capsule form. Each capsule, containing 300 mg of ginger extract with 15 mg of active ingredient per capsule (60 mg per 1.2 g), was double encapsulated to enhance patient blinding. Placebos were prepared with an inert filler and capsules that matched the intervention. De-identified supplements were randomised by an independent company prior to delivery to the recruitment sites. All staff members involved in recruitment were blinded to the results of randomisation. The gingerol and shogaol content of the ginger extract were independently analysed at the beginning and end of the trial by Southern Cross Plant Science Department at Southern Cross University and Bond University, respectively, using high performance liquid chromatography to ensure consistent potency of the intervention.

9.3.3 *Procedure*

Patients were randomised into intervention or placebo groups using a computer generated randomisation sequence. Randomisation was undertaken by investigators who had no contact with participants. Participants were followed over three chemotherapy cycles in order to obtain results that reflected their experience of ginger supplementation and CINV over an extended period of their chemotherapy treatment. For each cycle, outcomes were assessed 3 days prior to chemotherapy until

4 days post-chemotherapy (i.e. over 7 days). Participants were asked to consume the study capsules 4 times per day, with each meal, for 5 days per chemotherapy cycle, commencing on the day of chemotherapy.

9.3.4 *Outcome measures*

9.3.4.1 Primary outcome

The primary outcome was chemotherapy-induced nausea-related (CIN) quality of life (QoL). This was measured using the Functional Living Index Emesis 5 Day Recall (FLIE-5DR) questionnaire, a validated measure of the impact of CINV on patients' general well-being.[17] It comprises eighteen 7-point Likert scales that assess the separate effects of nausea and vomiting on QoL. Scores can range from 9 to 63 for each domain (i.e. nausea or vomiting) and 18 to126 for the total CINV score. A higher score indicates better QoL. To the authors' knowledge, no minimal clinically important difference has been established for the FLIE-5DR; however, using the parameters established by Martin et al,[18] "no impact on daily life" was defined as an average item score greater than 6 on the 7-point scale. Therefore, a total score greater than 108 (out of a total score of 126) and a domain-specific score of 54 (out of a total score of 63) meant that CINV had minimal impact on daily life. Participants completed this questionnaire twice per chemotherapy cycle, at baseline and 4 days post-chemotherapy.

9.3.4.2 Secondary outcomes

A total score for CINV as well as separate scores for nausea, retching and vomiting were elicited using the validated, 8-item self-report tool, the Rhodes

Inventory of Nausea, Vomiting and Retching (INVR).[19] The INVR assesses the frequency, duration and severity of nausea, vomiting and retching. It provides domain-specific scores for nausea, vomiting and retching as well as a total score for CINV calculated from the combined domains.

The following operational definitions defined each phase of CINV. Anticipatory CINV was defined as any symptom occurring in the 24 hours prior to chemotherapy administration.[20] Acute CINV was defined as any nausea and/or vomiting symptoms that occurred within 24 hours of the administration of chemotherapy, while delayed CINV was defined as any nausea and/or vomiting symptoms that occurred after the acute phase and for the following 5 days.[20] In order to measure each of these phases of CINV, the INVR was administered one day before the commencement of chemotherapy (anticipatory CINV), on the day of chemotherapy (acute CINV) and during each of the 4 proceeding days to assess delayed CINV.

The INVR is designed to measure symptoms over a 12 hour period; however, to minimise survey burden this period was extended to 24 hours so that participants would only need to complete one questionnaire per 24 hours. For each 24 hour period, a score (between 3-15 for nausea and vomiting, 2-10 for retching) is given for each symptom and a total score is derived from each symptom score (between 8-40). For delayed symptoms, scores from the three 24 hour periods after the acute phase were combined. If a participant reported no experience with a symptom, this was considered a "complete response".

Nutritional status was assessed once per chemotherapy cycle, on the day of chemotherapy, by an appropriately-trained research dietitian (WM and LF) using the Patient Generated Subjective Global Assessment (PG-SGA) tool.[21] The PG-SGA provides a global rating of either A (well nourished), B (suspected or moderately malnourished) or C (severely malnourished), as well as a continuous score that increases with the severity of symptoms and the concomitant need for symptom management.

Global cancer-related QoL and cancer-related fatigue were assessed at baseline and 4 days post-chemotherapy using the Functional Assessment of Cancer Therapy-Global (FACT-G) and the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) assessment questionnaires, respectively.[22,23] Both questionnaires are valid and widely-used within the cancer setting. The FACT-G is a self-report questionnaire that contains 27 five-point Likert scales that assess four domains of global (as opposed to CINV-specific) QoL. These are physical well-being, social/family well-being, emotional wellbeing, and functional well-being. The FACIT-F comprises 13 five point Likert scales, and was used to capture self-reported symptoms of fatigue before and after each chemotherapy cycle. Possible scores for the FACT-G and FACIT-F range from 0-108 and 0-52, respectively, with higher scores indicating better QoL and less fatigue. A four-point difference between groups in FACT-G scores was considered a clinically meaningful difference.[24] Participants were deemed clinically fatigued if they reported a FACIT-F score $\leq$34, with a difference of 3 points between groups considered a clinically meaningful difference in levels of fatigue.[25,26] Exercise was not monitored and no guidelines regarding physical activity were provided to participants.

A new questionnaire was developed as part of this project to assess the prevalence of prognostic factors that previous studies had identified increased the risk of CINV. The rationale for implementing this questionnaire was that an uneven distribution of these prognostic factors between the intervention and placebo groups could influence the results of this study. The questionnaire included five items that assessed the patient's history of morning sickness and motion sickness, their average weekly alcohol intake and history of anxiety.[27-29] Additional prognostic factors including age, gender and emetogenicity of the chemotherapy regimen were retrieved during the initial patient interview.

To determine participant adherence to the study protocol, participants were asked to record the number of capsules consumed each day during the study period. The quality of patient blinding was assessed at the end of each chemotherapy cycle during participant interviews, in which participants were asked to state the capsule (placebo or ginger) they believed they had received.

9.3.5 *Adverse events*

Safety concerns and adverse events were monitored via telephone interviews during each cycle, five days post-chemotherapy. Participants were asked about any hospitalizations or adverse events during the study period. To assess possible negative effects of ginger supplementation, between-group differences in a range of symptoms were also assessed using the Edmonton Symptom Assessment Scale, which was administered each cycle, at baseline and 5 days post-chemotherapy.[30] This is a validated 10-item questionnaire that measures the severity of common symptoms experienced by cancer patients including pain, anxiety and drowsiness.

9.3.6 *Statistical analysis*

Statistical analysis was conducted using SPSS Version 20®. Descriptive analysis of baseline participant characteristics was undertaken. Bivariate outcomes were assessed using chi-square analysis. Normally distributed continuous outcomes were assessed using independent sample t-tests. The Mann-Whitney U test was used for non-parametric outcomes. Prognostic factors were also assessed in this fashion. Data were analysed on an intention-to-treat basis. A *P* value <0.05 was deemed to indicate a statistically significant difference. Mean and standard deviation (SD) were reported for normally distributed data. Median and 25th percentile and 75th percentile were reported for non-parametric data. Missing data were handled using multiple imputation. In order to explore the association between ginger supplementation combined with aprepitant and worse delayed-CINV, a subgroup analysis in patients receiving aprepitant was also conducted.

Sample size was calculated based on the ability to detect a clinically meaningful difference in the primary outcome of nausea-related QoL. Hence, using the standard deviations from a preliminary feasibility study of ten participants and a desired mean difference of 9 points on the nausea-related subdomain of the FLIE-5DR, a sample size of 64 was estimated to provide sufficient power to detect a statistically and clinically significant difference in nausea-related QoL with 80% power and 5% significance. An additional 20% allowance was added to this sample size to allow for attrition, resulting in a final sample size of 77 participants.

9.4 Results

Table 9-1 Patient demographics at baseline

	Total	Intervention Group	Control Group
n	51	24	27
Age (mean±sd, years)	58±12	57±14	59±11
Gender (n, %female)	32 (63)	16 (66)	16 (59)
Race (n, %Caucasian)	42 (82)	18 (75)	24 (88)
Primary diagnoses			
Breast	13	7	6
Colon	19	8	11
Lymphoma	11	5	6
Other	8	4	4
Chemotherapy Emetogenicity			
HEC	8.0	4.0	4.0
MEC	43.0	20.0	23.0
Receiving aprepitant	18.0	7.0	11.0

HEC=Highly Emetogenic Chemotherapy. MEC=Moderately Emetogenic Chemotherapy. These regimens were classified based on the Multinational Association of Supportive Care in Cancer guidelines. [31]

Patient demographics and adherence

Fifty-one patients were enrolled in this study, of which 34 completed all three cycles (Figure 9-1). There were no significant differences in baseline patient characteristics between the intervention and placebo group ($p > 0.05$). The majority of patients (85%) were scheduled to undergo moderately emetogenic chemotherapy regimens (Table 9-1).

9.4.1 *CINV- related Quality of Life*

After cycle 1, participants assigned to the intervention group reported better nausea-related QoL (Median [25th, 75th percentile] = 61.5 [56.1, 63] vs 54 [46, 63]; p=0.029) and better total CINV-related QoL (Median [25th, 75th percentile] = 124.5 [113, 126] vs 111 [99.2, 126]; p=0.043) compared to patients assigned the placebo. Examination of median CINV- and nausea- related QoL at cycle 1 in the placebo and intervention groups suggests that the clinically significant effect of total CINV and nausea on QoL was minimal in both groups (Table 9-2). No other significant effect was detected for vomiting-related QoL or for any outcome at cycles 2 and 3.

9.4.2 *Nausea and vomiting symptoms*

Over the three chemotherapy cycles, acute and delayed CINV occurred in 39% and 65% of all participants (Table 9-3). In both groups, nausea was more common than vomiting during each cycle, with 47% vs 12% of participants overall reporting symptoms during at least one cycle, respectively. There were no significant differences in the prevalence and score of CINV between the intervention and placebo group at any time point.

In a subgroup analysis of participants (n=18) assigned to the intervention with and without being prescribed aprepitant, there were no statistically significant differences in CINV between groups at any time point (Table 9-4).

9.4.3 *Fatigue, nutrition status, and cancer-related quality of life*

Clinically significant fatigue and malnutrition were experienced by 36% and 22% of participants over the study period. Ginger supplementation was associated

with improved measures of chemotherapy-related fatigue in cycles 1 (Mean±SD = 41.8±13.1vs 32.2±10.8; p=0.001) and cycle 3 (Mean±SD = 42.4±10.2 vs 36.1±7.2; p=0.013) compared to placebo. There was also a statistically significant difference in cancer-related QoL at cycle 1 (Mean±SD = 83.6±15.0 vs 75.1±13.9; p=0.015) and cycle 3 (Mean±SD = 85.1±18.9 vs 71.9±18.3; p=0.040).

Each of the significant associations reported for the cancer-related QoL (>4 point difference) and cancer-related fatigue (>3 point difference) were also clinically significant differences. No significant difference in nutritional status was detected between the intervention and placebo group during the study period (p>0.05; Table 9-2).

9.4.4 *Participant blinding and adherence*

More participants in the intervention group were able to correctly guess their assigned group when compared to participants in the placebo group (63% compared to 30%, respectively). For participants in the intervention group who successfully identified their allocation, the most common rationale provided was a lack of nausea (60%), the smell of the capsules (20%), and ginger taste or reflux (10%). Adherence to the study intervention was moderate-to-high, with 70% of all participants (69% in ginger group and 75% in placebo group) consuming at least 3 of the 4 prescribed capsules per day.

Table 9-2 Cancer- and CINV-related Qol, cancer-related fatigue, and nutrition status

	Cycle 1				**Cycle 2**				**Cycle 3**			
	Total	Placebo	Intervention	*P* value	Total	Placebo	Intervention	*P* value	Total	Placebo	Intervention	*P* value
CINV-QoL	124 [103, 126]	111 [99.2, 126]	124.5 [113, 126]	0.043*	124 [108, 126]	117 [109, 126]	124 [107, 126]	0.916	122 [107, 126]	120 [111, 126]	123 [107, 126]	0.931
Complete response n(%)	37 (73)	17 (63)	20 (83)	0.104	40 (78)	22 (81)	18 (75)	0.574	38 (75)	21 (78)	17 (71)	0.57
Nausea-related QoL	60 [50.7, 63]	54 [46, 63]	61.5 [56.1, 63]	0.029*	61 [49, 63]	55.6 [48.7, 63]	61 [52.1, 63]	0.494	56 [48.9, 63]	56 [48.9, 63]	56.5 [47, 63]	0.931
Complete response n(%)	33 (65)	14 (52)	19 (79)	0.042*	30 (59)	15 (56)	15 (63)	0.615	31 (61)	16 (59)	15 (63)	0.813
Vomiting-related QoL	63 [51.3, 63]	63 [50.7, 63]	63 [54.2, 63]	0.237	63 [51.9, 63]	63 [51.7, 63]	63 [54.4, 63]	0.663	63 [50.6, 63]	63 [53.6, 63]	58.9 [50.1, 63]	0.414
Complete response n(%)	37 (73)	19 (70)	18 (75)	0.712	37 (73)	19 (70)	18 (75)	0.712	34 (67)	20 (74)	14 (58)	0.234
Global cancer-related QoL	77.4±19.5	71.9±18.3	85.1±18.9	0.015*	70±14.8	67.6±10.2	74.9±17.7	0.075	79.3±15.1	75.1±13.9	83.6±15.0	0.040*
Fatigue	36.2±12.8	32.2±10.8	41.8±13.1	0.007*	36.1±9.6	34.5±7.9	37.7±10.8	0.231	39.1±9.3	36.1±7.2	42.4±10.2	0.013*
Nutrition status at start of cycle (n well nourished)	44	22	22	0.371	38	19	19	0.500	37	19	18	0.622

Total response was defined as a score > 54 on the nausea- and vomiting- related quality of life scores and a score >108 on the chemotherapy-induced nausea and vomiting-related quality of life score
Normally distributed measures were presented as mean±standard deviation and non-normally distributed measures were presented as median [25th percentile, 75th percentile]

Table 9-3 Participant INVR questionnaire scores and CINV prevalence

	Cycle 1				**Cycle 2**				**Cycle 3**			
	Total	Placebo	Intervention	*p* value	Total	Placebo	Intervention	*p* value	Total	Placebo	Intervention	*p* value
Anticipatory CINV score	8 [8,8]	8 [8,8]	8 [8,8]	0.49	8 [8,8.8]	8 [8,8.8]	8 [8,8.8]	0.40	8 [8,8.4]	8 [8,8.6]	8 [8,8.5]	0.23
Acute CINV score	9.93 [8.65,10.87]	8 [8,9.4]	9.6 [8,10]	0.78	9.02 [8.5,9.6]	8 [8,9.6]	8.57 [8,9.52]	0.90	9.89 [8.65, 10.87]	8 [8,9.4]	8.8 [8,10.1]	0.17
Complete response n(%)	30 (59)	17 (63)	13 (54)	0.49	33 (65)	18 (67)	15 (63)	0.90	31 (61)	17 (63)	14 (58)	0.75
Vomiting score	3 [3,3]	3 [3,3]	3[3,3.1]	0.79	3 [3,3]	3 [3,3]	3 [3,3]	1.00	3 [3,4]	3 [3,3.2]	3 [3,5.1]	0.31
Complete response n(%)	47 (92)	25 (93)	22 (92)		51 (100)	27 (100)	24 (100)	1.00	43 (84)	24 (89)	19 (79)	0.46
Nausea score	3 [3,5]	3 [3,4.8]	3.6 [3,5]	0.38	3.29 [3,4.33]	3 [3,4.27]	3.8 [3, 4.45]	0.62	3.2 [3,5]	3 [3,5]	3[3,4.8]	0.74
Complete response n(%)	33 (65)	19 (70)	14 (58)	0.47	33 (65)	18 (67)	15 (63)	0.90	35 (69)	19 (70)	16 (67)	0.64
Retching score	2 [2,2]	2 [2,2]	2 [2,2.1]	0.75	2 [2,2]	2 [2,2]	2 [2,2]	1.00	2 [2,2]	2 [2,2]	2 [2,2.1]	0.30
Delayed CINV score	31.31 [27.4,34.4]	32 [24,35]	26 [24,34.3]	0.72	29.5 [28.23,31.07]	28.95 [24, 33]	28.67 [24,29.95]	0.24	28.81 [27.13,30.35]	27.4 [24,31.8]	28 [24,30.6]	0.90
Complete response n(%)	14 (27)	6 (22)	8 (33)	0.53	23 (45)	9 (33)	7 (29)	0.83	17 (33)	10 (37)	7 (29)	0.62
Vomiting score	9 [9,10.11]	9 [9,11.8]	9 [9,9.7]	0.97	9 [9,9.89]	9 [9,10.17]	9 [9,9.88]	0.97	9 [9,9.40]	9 [9,9.66]	9 [9,9.4]	0.93
Complete response n(%)	41 (80)	21 (78)	20 (83)	0.75	42 (82)	22 (81)	20 (83)	1.00	46 (90)	24 (89)	22 (92)	0.50
Nausea score	13 [9,19]	15 [9,20]	11 [9,17.9]	0.27	13 [9,15.48]	12.39 [9,15.88]	14.99 [9,15.78]	0.49	12 [9,16.32]	11.95 [9,15.77	12 [9,16.43]	0.39
Complete response n(%)	20 (39)	9 (33)	11 (46)	0.61	19 (37)	11 (41)	8 (33)	0.50	23 (45)	14 (52)	9 (38)	0.42
Retching score	6 [6,6]	6[6,6]	6 [6,6.05]	0.40	6 [6,6.4]	6 [6,6.4]	6 [6,6.5]	0.91	6 [6,6]	6 [6,6]	6 [6,6]	0.89

Normally distributed measures were presented as mean±standard deviation and non-normally distributed measures were presented as median [25th percentile, 75th percentile]

Table 9-4 Sub-group analysis of INVR scores of participants prescribed aprepitant

	Cycle 1			Cycle 2			Cycle 3		
	Placebo	**Intervention**	***p* value**	**Placebo**	**Intervention**	***p* value**	**Placebo**	**Intervention**	***p* value**
Acute CINV score	8 [8,9]	10 [8,23]	0.108	8[8,10.2]	8.8 [8,10]	0.449	8 [8,10]	10 [8, 13.2]	0.247
Vomiting score	3[3,3]	3[3,8.2]	0.067	3[3,3]	3[3,3]	1	3 [3,4.5]	3 [3,5.1]	0.823
Nausea score	3[3,4]	5[3,9.4]	0.191	3[3,5.2]	3.8[3,5]	0.449	3[3,4.5]	4.8 [3,5]	0.393
Delayed CINV score	24[24,26.5]	34[25,40.3]	0.069	28 [24,38.2]	32[24,41.7]	0.686	28 [25,44]	33[24,43.7]	0.929
Vomiting score	9[9,9]	9[9,14.3]	0.067	9[9,9]	9[9,13.3]	0.105	3 [3,3]	3[3,4]	0.332
Nausea score	9[9,11.5]	15[10.5,19.5]	0.068	13[9,24.5]	17[9,20.3]	0.857	4 [3.1,6.5]	4.4[3,5.5]	0.752

Normally distributed measures were presented as mean±standard deviation and non-normally distributed measures were presented as median [25th percentile, 75th percentile]

9.4.5 *Effect of prognostic factors on CINV-related outcomes*

The hypothesised prognostic factors of age, gender, anticipatory CINV, and chemotherapy emetogenicity were analysed with no significant associations detected ($p>0.05$) between these variables and any measure of CINV.

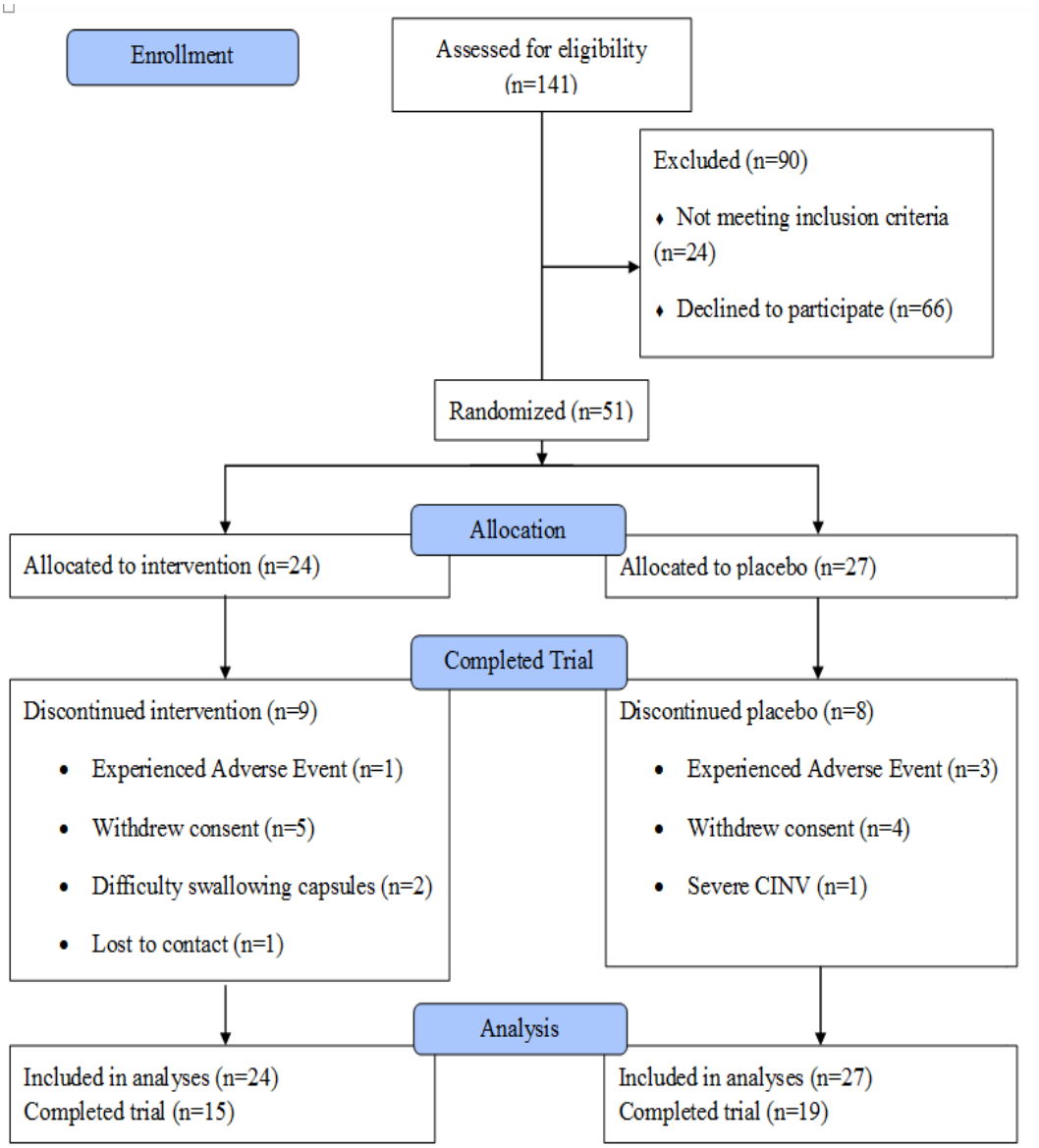
9.4.6 *Adverse events*

Four patients in this trial experienced significant adverse events, none of which could reasonably be attributed to the ginger intervention. These include one participant whose lung collapsed, one allergic reaction to pegfilgrastim, and two emergency room admissions due to neutropenic fever. Three of the four adverse events occurred within the placebo group. The most commonly reported side-effects in the intervention group included constipation and reflux, which were reported by two and four participants, respectively.

9.4.7 *Incomplete questionnaires*

Few participants completed the Edmonton Symptom Assessment Scale and CINV-prognostic questionnaire hence the results from these questionnaires were not statistically meaningful.

Figure 9-1 CONSORT Flow Diagram



9.5 Discussion

The results of this study indicate that, compared to placebo, adjuvant ginger is associated with better nausea-related QoL, less cancer-related fatigue and better cancer-related QoL. Previous studies have reported that ginger reduces CINV; however, this is the first study to investigate whether this reduction translates into an improvement in

QoL. As CINV has been demonstrated to significantly reduce QoL,[17] this study provides evidence that ginger supplementation could be a viable adjuvant to traditional pharmacotherapy for CINV that enhances patient's wellbeing during their cancer treatment.

Despite the significant effect of ginger on QoL, the findings indicate there was no significant effect on the prevalence or severity of CINV. While the majority of previous research has reported that ginger supplementation reduces the incidence and severity of CINV, not all studies have reported benefits.[14,32] The prevalence of CINV during this study was high (39% and 65% of patients experienced acute and delayed CINV, respectively) which is consistent with the prevalence reported in other studies.[1] However, similar to the results of Fahimi et al,[32] while the prevalence was high, the average score derived from the INVR was low in both the intervention and placebo groups. This indicates that although a large proportion of participants experienced CINV, the average severity caused by these symptoms was low. Furthermore, the results of Ryan et al,[33] the largest RCT conducted in this area to date (N=576), reported minor improvements in acute nausea. This could suggest that statistically significant group differences in CINV severity in this study could not be detected with the relatively smaller sample size.

The statistically significant improvement in nausea-related QoL, could also be clinically relevant. However, as there is no established minimal clinically-important difference for the FLIE-5DR, the clinical significance of the better CINV- and nausea-related QoL in cycle 1 reported in the intervention group is not easily elucidated. A score

of 108 or greater was used in this study to indicate that CINV had no effect on QoL.[18] Using this cut-off, an examination of median CINV- and nausea- related QoL in the placebo group after cycle 1 (Median [25th, 75th percentile] = 111 [99.2, 126] and 54 [46, 63], respectively) revealed that the placebo group experienced poorer CINV-related QoL. While these results indicate a significant difference between groups, due to the generally high level of CINV- and nausea- related QoL in both intervention and placebo groups, it is difficult to determine the clinical significance of the ginger supplementation used in this trial with respect to CINV- and nausea- related QoL. Similarly high ratings of QoL have been reported in previous observational studies.[3,34] A possible explanation for this is that this trial was conducted at a hospital that adheres to international anti-emetic guidelines and prescribes current generation anti-emetic medications such as aprepitant and granisetron. In contrast, many previous studies that have reported severe CINV were conducted before the standard introduction of these anti-emetics into clinical practice.[35,36] Furthermore, the process of "response shift'" could have also influenced these results.[37] Response shift refers to the individual's re-evaluation of the internal standards and the values with which they assess their quality of life, a process associated with repeat experiences of their treatment and its symptoms or comparison with other people's experiences of it, which can appear comparatively worse than their own.[37] CINV-related QoL within the placebo group gradually improved (Table 9-2) which suggests that a "response shift" in participant's assessment of QoL could have occurred over the course of their chemotherapy treatment.

The significant improvement in fatigue reported in this study corroborates the results by Zick et al.[38], who reported that fatigue was the most common adverse event in the placebo group but not in the ginger intervention group. While the exact mechanism underpinning this finding is unknown, future studies could investigate the role of ginger in cancer-related fatigue.

Ginger supplementation was well-tolerated with no significant increase in adverse events and few side-effects reported. This is consistent with previous studies, which have reported minor adverse events.[7] Ginger has been reported in some (but not all) clinical studies to interfere with platelet aggregation.[39] During chemotherapy, this can potentially pose a significant concern due to the pre-existing risk of thrombocytopenia. Although there has been no indication of adverse clotting in this trial or previous studies, platelet function should be routinely monitored in this patient group.[39]

Another potential concern is that ginger might reduce the effectiveness of anti-emetic therapy when patients are prescribed aprepitant. This was identified in a subgroup analysis in one study, which reported that patients who received 2g of ginger and aprepitant experienced worse delayed CINV than patients who received 2g of ginger without aprepitant.[14] This association, however, was not identified in patients prescribed aprepitant and a lower dose of ginger (1g) indicating that this might only occur with higher doses of ginger (2g).[14] In the present study, participants receiving ginger supplementation and aprepitant reported worse control of delayed CINV (Table 9-4); however, this difference was neither statistically nor clinically significant. This

relationship should continue to be investigated in larger trials to ensure that patients' anti-emetic control is not compromised by ginger supplementation.

Despite our ginger supplements being doubly encapsulated, the most commonly reported side-effect in the intervention group was ginger taste or reflux. While this was considered a mild side-effect by participants, it is a potential confounding variable for clinical trials investigating ginger as the unique taste is likely to reduce the efficacy of blinding, which could influence subjective measures of nausea. In order to improve blinding efficacy, testing of blinding efficacy in a small sample before the commencement of recruitment is recommended.

This study has overcome limitations identified in previous studies by including the use of standardized extracts and chemical analysis of supplements that ensured potency throughout the study period, as well as the assessment of previously identified prognostic factors such as age and gender. Previous studies have not assessed the influence of these prognostic factors, which might have resulted in an imbalanced risk of CINV between the two treatment arms in these studies. Furthermore, in this study we controlled for anticipatory CINV, a conditioned response that develops via a pathway different to other types of CINV.[29] This was achieved by recognising that the prevalence of anticipatory CINV increases with subsequent cycles; hence only chemotherapy-naïve patients were recruited.[40] CINV was also assessed the day before each cycle of chemotherapy to capture anticipatory CINV. None of these prognostic factors were found

to be associated with CINV-related outcomes, which could be linked to the small sample size.

A further strength of this study was the four-per-day dosing regimen in contrast to the once or twice per day regimens adhered to in previous studies, which accounted for the relatively short half-life of major ginger compounds.[41,42] Based on our understanding of the pharmacokinetics of ginger, we hypothesised that more frequent consumption of ginger would ensure sufficient plasma levels of the active compounds which could result in a greater level of effect.[41] Future studies that include additional arms are needed to determine the effect of different dosing regimens on the treatment effect.

We acknowledge the following limitations. First, there was a high level of attrition after cycle 3 (33%) compared to previous studies, which have reported an attrition rate of approximately 20%,[32,33,38] Due to the extended study time frame (3 cycles compared to 1-2), the increased number of capsules ingested required per day, and the expanded number of outcomes that were measured, the relatively high attrition could be because the trial protocol was overly burdensome to participants. This is also demonstrated by the low completion rates of some questionnaires (CINV prognostic and ESAS questionnaires) which prevented meaningful statistical analysis of these outcomes. In addition, 75% (6/8) of participants prescribed highly-emetogenic chemotherapy regimens had withdrawn by cycle 3, compared to 25% of patients receiving moderately-emetogenic chemotherapy regimens. This suggests that participants undergoing more emetogenic regimens might have had difficulty completing the added duties required of participation

within this study for reasons not related to CINV, such as other symptoms. In future studies, it is recommended that particular consideration is taken to reduce the burden that is placed on participants by the study protocol such as by reducing the number of self-reported outcome measures.

Another limitation was that, while this trial was sufficiently powered to detect a significant difference in the primary outcome at cycle 1, due to timing constraints and attrition, the trial did not meet the required sample size for the second and third cycles. Introducing inflation factors in sample size calculations, as well as reducing study burden, is recommended in future studies to ensure sufficient power during subsequent cycles.

9.6 Conclusion

In summary, the results of this clinical trial suggest that compared to placebo, adjuvant ginger is associated with better chemotherapy-induced nausea-related and cancer-related quality of life, and less cancer-related fatigue. The results confirm several previous studies that report ginger supplementation to be well-tolerated and without significant side-effects. Further studies with larger sample sizes are required to confirm these results and to further explore the safety profile of ginger supplementation during chemotherapy.

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9.8 References

1. Hsieh RK, Chan A, Kim HK, et al. Baseline patient characteristics, incidence of CINV, and physician perception of CINV incidence following moderately and highly emetogenic chemotherapy in Asia Pacific countries. *Support Care Cancer.* 2015;23(1):263-272.
2. Davidson W, Teleni L, Muller J, et al. Malnutrition and chemotherapy-induced nausea and vomiting: implications for practice. *Oncol Nurs Forum.* 2012;39:E340 - 345.
3. Bloechl-Daum B, Deuson RR, Mavros P, Hansen M, Herrstedt J. Delayed nausea and vomiting continue to reduce patients' quality of life after highly and moderately emetogenic chemotherapy despite antiemetic treatment. *J Clin Oncol.* 2006;24(27):4472-4478.
4. Neymark N, Crott R. Impact of emesis on clinical and economic outcomes of cancer therapy with highly emetogenic chemotherapy regimens: a retrospective analysis of three clinical trials. *Support Care Cancer.* 2005;13(10):812-818.
5. Molassiotis A, Yung HP, Yam BM, Chan FY, Mok TS. The effectiveness of progressive muscle relaxation training in managing chemotherapy-induced nausea and vomiting in Chinese breast cancer patients: a randomised controlled trial. *Support Care Cancer.* 2002;10(3):237-246.

6. Srivastava M, Brito-Dellan N, Davis M, Leach M, Lagman R. Olanzapine as an antiemetic in refractory nausea and vomiting in advanced cancer. *J Pain Symptom Manage.* 2003;25:578 - 582.

7. Marx WM, Teleni L, McCarthy AL, et al. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. *Nutr. Rev.* 2013;71(4):245-254.

8. Marx W, Ried K, McCarthy AL, et al. Ginger-Mechanism of Action in Chemotherapy-induced Nausea and Vomiting: A Review. *Crit Rev Food Sci Nutr.* 2015:0.

9. Marx W, Kiss N, Isenring L. Is ginger beneficial for nausea and vomiting? An update of the literature. *Current Opinion in Supportive and Palliative Care.* 2015;9(2):189-195.

10. Chaiyakunapruk N, Kitikannakorn N, Nathisuwan S, Leeprakobboon K, Leelasettagool C. The efficacy of ginger for the prevention of postoperative nausea and vomiting: a meta-analysis. *Am J Obstet Gynecol.* 2006;194(1):95-99.

11. Viljoen E, Visser J, Koen N, Musekiwa A. A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting. *Nutr J.* 2014;13:20.

12. Marx W, McCarthy A, Ried K, et al. Can ginger ameliorate chemotherapy-induced nausea? Protocol of a randomized double blind, placebo-controlled trial. *BMC Complementary and Alternative Medicine.* 2014;14(1):134.

13. Oh H, Seo W. Systematic review and meta-analysis of the correlates of cancer-related fatigue. *Worldviews Evid Based Nurs.* 2011;8:191 - 201.

14. Zick SM, Ruffin MT, Lee J, et al. Phase II trial of encapsulated ginger as a treatment for chemotherapy-induced nausea and vomiting. *Support Care Cancer.* 2009;17(5):563-572.

15. Schag CC, Heinrich RL, Ganz PA. Karnofsky performance status revisited: reliability, validity, and guidelines. *J Clin Oncol.* 1984;2(3):187-193.

16. Roila F, Hesketh PJ, Herrstedt J. Prevention of chemotherapy- and radiotherapy-induced emesis: results of the 2004 Perugia International Antiemetic Consensus Conference. *Ann Oncol.* 2006;17(1):20-28.

17. Lindley CM, Hirsch JD, O'Neill CV, Transau MC, Gilbert CS, Osterhaus JT. Quality of life consequences of chemotherapy-induced emesis. *Qual Life Res.* 1992;1(5):331-340.

18. Martin AR, Pearson JD, Cai B, Elmer M, Horgan K, Lindley C. Assessing the impact of chemotherapy-induced nausea and vomiting on patients' daily lives: a modified version of the Functional Living Index-Emesis (FLIE) with 5-day recall. *Support Care Cancer.* 2003;11(8):522-527.

19. Rhodes VA, McDaniel RW. The Index of Nausea, Vomiting, and Retching: a new format of the lndex of Nausea and Vomiting. *Oncol Nurs Forum.* 1999;26(5):889-894.

20. National Comprehensive Cancer Network (NCC). NCCN Practice Guidelines in Oncology™ [v.1.2015]: Antiemesis. 2015; http://www.nccn.org/professionals/physician_gls/pdf/antiemesis.pdf.

21. Isenring E, Bauer J, Capra S. The scored Patient-generated Subjective Global Assessment (PG-SGA) and its association with quality of life in ambulatory patients receiving radiotherapy. *Eur J Clin Nutr.* 2003;57(2):305-309.

22. Cella D, Hahn EA, Dineen K. Meaningful change in cancer-specific quality of life scores: differences between improvement and worsening. *Qual Life Res.* 2002;11(3):207-221.

23. Yellen SB, Cella DF, Webster K, Blendowski C, Kaplan E. Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. *J Pain Symptom Manage.* 1997;13(2):63-74.

24. Cella D, Eton DT, Lai JS, Peterman AH, Merkel DE. Combining anchor and distribution-based methods to derive minimal clinically important differences on the Functional Assessment of Cancer Therapy (FACT) anemia and fatigue scales. *J Pain Symptom Manage.* 2002;24(6):547-561.

25. Van Belle S, Paridaens R, Evers G, et al. Comparison of proposed diagnostic criteria with FACT-F and VAS for cancer-related fatigue: proposal for use as a screening tool. *Support Care Cancer.* 2005;13(4):246-254.

26. Webster K, Cella D, Yost K. The Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System: properties, applications, and interpretation. *Health and Quality of Life Outcomes.* 2003;1:79-79.

27. Persistence of efficacy of three antiemetic regimens and prognostic factors in patients undergoing moderately emetogenic chemotherapy. Italian Group for Antiemetic Research. *J Clin Oncol.* 1995;13(9):2417-2426.

28. Hickok JT, Roscoe JA, Morrow GR. The Role of Patients' Expectations in the Development of Anticipatory Nausea Related to Chemotherapy for Cancer. *J Pain Symptom Manage.* 2001;22(4):843-850.

29. Pirri C, Katris P, Trotter J, Bayliss E, Bennett R, Drummond P. Risk factors at pretreatment predicting treatment-induced nausea and vomiting in Australian cancer patients: a prospective, longitudinal, observational study. *Support Care Cancer*. 2011;19(10):1549-1563.

30. Chang VT, Hwang SS, Feuerman M. Validation of the Edmonton Symptom Assessment Scale. *Cancer.* 2000;88(9):2164-2171.

31. Roila F, Herrstedt J, Aapro M, et al. Guideline update for MASCC and ESMO in the prevention of chemotherapy- and radiotherapy-induced nausea and vomiting: results of the Perugia consensus conference. *Ann Oncol.* 2010;21(5):v232-v243.

32. Fahimi F, Khodadad K, Amini S, Naghibi F, Salamzadeh J, Baniasadi S. Evaluating the Effect of Zingiber Officinalis on Nausea and Vomiting in Patients Receiving Cisplatin Based Regimens. *Iran J Pharm Res.* 2011;10(2):379-384.

33. Ryan JL, Heckler CE, Roscoe JA, et al. Ginger (Zingiber officinale) reduces acute chemotherapy-induced nausea: a URCC CCOP study of 576 patients. *Support Care Cancer.* 2012;20(7):1479-1489.

34. Cohen L, de Moor CA, Eisenberg P, Ming EE, Hu H. Chemotherapy-induced nausea and vomiting: incidence and impact on patient quality of life at community oncology settings. *Support Care Cancer*. 2007;15(5):497-503.

35. Sontakke S, Thawani V, Naik MS. Ginger as an antiemetic in nausea and vomiting induced by chemotherapy: A randomized, cross-over, double blind study. *Indian J. Pharmacol.* 2003;35(1):32-36.

36. Manusirivithaya S, Sripramote M, Tangjitgamol S, et al. Antiemetic effect of ginger in gynecologic oncology patients receiving cisplatin. *Int J Gynecol Cancer.* 2004;14(6):1063-1069.

37. Sprangers MAG, Schwartz CE. Integrating response shift into health-related quality of life research: a theoretical model. *Social Science & Medicine.* 1999;48(11):1507-1515.

38. Zick S, Ruffin M, Lee J, et al. Phase II trial of encapsulated ginger as a treatment for chemotherapy-induced nausea and vomiting. *Supportive Care in Cancer.* 2009;17(5):563-572.

39. Marx W, McKavanagh D, McCarthy AL, et al. The Effect of Ginger (Zingiber officinale) on Platelet Aggregation: A Systematic Literature Review. *PLoS ONE.* 2015;10(10):e0141119.

40. Petrella T, Clemons M, Joy A, Young S, Callaghan W, Dranitsaris G. Identifying Patients at High Risk for Nausea and Vomiting After Chemotherapy: The Development of a Practical Validated Prediction Tool. *J Support Oncol* 2009;7(4):9-16.

41. Yu Y, Zick S, Li X, Zou P, Wright B, Sun D. Examination of the pharmacokinetics of active ingredients of ginger in humans. *AAPS J.* 2011;13(3):417-426.

42. Zick SM, Djuric Z, Ruffin MT, et al. Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev.* 2008;17(8):1930-1936.

Chapter 10. The attitudes, beliefs and behaviours of Dietitians and Health Care Professionals regarding dietary supplements.

This chapter presents the results of a survey that investigated the attitudes, beliefs and behaviours of 370 healthcare professionals regarding dietary supplements. The results of this survey were presented at the following conference:

Wolfgang Marx, Nicole Kiss, Daniel McKavanagh, Liz Isenring. Chemotherapy-induced nausea and vomiting: a guide for dietetic practice. *Clinical Oncology Society of Australia* (17–19 November 2015, Hobart, Australia).
Oral symposium presentation

Citation*:* **Marx WM**, Kiss N, McKavanagh D, Isenring E. The attitudes, beliefs and behaviours of healthcare professionals regarding dietary supplements. BioMed Central Alternative and Complementary Medicines. Impact factor: 2.02; Intended submission: December, 2015

Relevant Appendices: Appendices F. Survey Questions Plan

10.1 Abstract

Due to the high prevalence of dietary supplement use, there is a potential for misinformation, underestimation of side-effects, and drug-nutrient interactions. Therefore, it is pertinent to ask if healthcare professionals should play a greater role in the research, prescription, and education regarding dietary supplements. The aim of this study was to investigate the usage of dietary supplements in healthcare practise, barriers and enablers for their use, and the level of research interest and general knowledge by health practitioners.

An advertisement to an online survey was disseminated through the mailing lists of multiple healthcare organisations. There were 370 healthcare professionals that replied to the survey. The majority of respondents were dietitians, accounting for 78% of responses.

The results indicate that healthcare professionals are interested in dietary supplements (65%); however, due to the large number of barriers and 50% saying they do not regularly recommend dietary supplements (25% agreed, 25% neutral), the results also indicate that health care professionals are tentative about integrating dietary supplements into their clinical practice. Concerns regarding potential interactions with other treatments were reported as the number one barrier (67%) to utilizing dietary supplements as part of clinical practice. In addition, there was a strong interest in additional training in dietary supplements (81%), as well as the majority of respondents reporting that the current level of tertiary training in this area is inadequate (58%).

In summary, healthcare professionals are interested in the use of dietary supplements; however, due to current barriers, particularly concerns regarding drug-nutrient interactions, few healthcare professionals utilize dietary supplements as part of clinical practice. The results indicate that further research and training is required to address current knowledge deficits.

10.2 Introduction

The use of dietary herbal and vitamin supplements to treat or prevent chronic diseases has gained considerable interest both in academic research and amongst the general public. A large proportion of the population regularly use dietary supplements to help manage chronic conditions (e.g. arthritis, osteoporosis and heart health).[1] Up to 73% of the general public within the United States consume dietary supplements and similar trends have been reported in other western counties.[2] Hence, the rapid uptake of supplements by the public has created the potential for misinformation, underestimation of side-effects, and drug-nutrient interactions. There have been numerous studies that have highlighted the potential risks associated with inappropriate use of dietary supplements. In a study of 171 patients who were recently prescribed warfarin, 43% were found to be taking dietary supplements that have previously been reported to interact with anticoagulation therapy.[3] Toxicity associated with inappropriate use of dietary supplements has been documented in numerous case-reports.[4,5] There is also evidence that the long-term use of antioxidant supplements may increase the risk of cancer in specific populations.[6] These issues are compounded by reports suggesting that a

significant percentage of certain patient populations are not discussing their use of dietary supplements with their physician.[7]

While the use of certain vitamin and mineral supplements have demonstrated negligible benefits in healthy populations, support for other supplements for primary prevention of chronic diseases is increasing.[8,9] Furthermore, the use of dietary supplements for specific conditions such as hypertension, antibiotic-associated diarrhoea, morning sickness, and in the critically ill have shown particular promise.[10-13]

Healthcare professionals are in a key position to advise patients and the general public about the evidence and limitations of specific dietary supplements. The aim of this study was to determine the usage of dietary supplements in healthcare practice, barriers and enablers for their use, and the level of research interest, confidence and general knowledge regarding dietary supplement-related issues. The results of this study will aid in elucidating existing gaps in knowledge and provide information on how dietary supplements are perceived and utilised in current practice.

10.3 Methods

The study sample was limited to healthcare professionals of any discipline who consult directly with patients and/or clients at the time of their participation in the study. Between August 2014 and August 2015, the survey was advertised online through the mailing lists and forums of the Dietitians Association of Australia, Dietitian Connection, the Multinational Association of Supportive Care and Cancer, and the Cancer Council Queensland. This study was approved by the Bond University Human Research Ethics Committee.

The survey format was designed and face validated by three senior dietitians in positions relevant to the study aim. The survey included 27 items to assess participants' attitudes towards specific issues related to dietary supplements (e.g. efficacy, safety, and feasibility/current usage), respondents' perception of professional and public perceptions, barriers and enablers for use, level of individual confidence and knowledge regarding dietary supplements. A pilot study (n=10) was conducted to detect feasibility issues with the survey. These issues were addressed in a revised version of the survey. Complete as well as partial responses were included in the results

For the purpose of this study, dietary supplements were defined as possessing the following characteristics: a vitamin, mineral, herb or other botanical, amino acid, or combination of those and/or other substances or constituents; intended to be ingested by mouth; and found in forms such as tablets, capsules, softgels, gelcaps, liquids, or powders. In order to limit responses to those that address the aims of this study, respondents were asked to disregard the following types of dietary supplements when completing the survey: high energy, high protein oral nutritional supplements used to treat malnutrition or undesired weight loss; and vitamin or mineral supplements used to correct diagnosed deficiencies caused by insufficient dietary intake in order to meet established recommended daily intakes.

10.4 Results

10.4.1 *Demographic*

There were 370 healthcare professionals that replied to the survey, of which there was complete data on 271 respondents. The majority of respondents were dietitians,

Table 10-1. Respondent demographics

	Total	Dietitians	Doctors	Nurses	Allied Health Professional	Miscellaneous
Responses	370	269(74)	23(6)	31(9)	20(6)	27
Age						
<30	138(38)	126(47)	0(0)	5(16)	5(25)	10(2)
31-40	90(25)	65(24)	8(35)	3(10)	8(40)	30(6)
41-50	59(16)	34(13)	5(22)	10(32)	5(25)	25(5)
51-60	60(17)	32(12)	8(35)	12(39)	2(10)	30(6)
>61	14(4)	10(4)	2(9)	1(3)	0(0)	5(1)
Years worked in current profession						
0-2	45(12)	42(16)	0(0)	0(0)	1(5)	10(2)
2-5	82(23)	75(28)	1(4)	0(0)	3(15)	15(3)
5-10	82(23)	59(22)	7(30)	6(19)	7(35)	15(3)
10-15	45(12)	32(12)	2(9)	2(6)	4(20)	25(5)
15-25	54(15)	30(11)	8(35)	11(35)	210)	15(3)
>25	55(15)	31(12)	5(22)	12(39)	3(15)	20(4)
Country of residence						
Australia	279(79)	228(87)	6(27)	23(77)	12(63)	67(10)
USA	24(7)	15(6)	3(14)	1(3)	1(5)	27(4)
Netherlands	8(2)	7(3)	0(0)	0(0)	0(0)	7(1)
Other	40(14)	11(4)	13(59)	6(21)	6(32)	0(0)
Highest level of education						
Diploma	18(5)	9(3)	1(4)	5(16)	0(0)	15(3)
Bachelor	167(46)	128(48)	2(9)	18(58)	12(60)	35(7)
Masters Degree	149(41)	124(46)	8(35)	5(16)	6(30)	30(6)
PhD	29(8)	8(3)	12(52)	3(10)	2(10)	20(4)
Area of practice						
Acute care	141(40)	102(39)	12(52)	9(29)	11(55)	35(7)
Community	99(28)	72(27)	6(26)	13(42)	3(15)	25(5)
Private Practice	67(19)	61(23)	1(4)	2(6)	2(10)	5(1)
Industry	6(2)	3(1)	0(0)	1(3)	1(5)	5(1)
Other	44(12)	25(10)	4(17)	6(19)	3(15)	30(6)

Data presented as n(%row)

accounting for 78% of responses. The next most common professions were nursing, medical and allied health professionals, accounting for 9%, 6% and 6% of responses, respectively. Other professions responded to the survey; however, due to the low response rate of some disciplines, they were not included in discipline-specific analysis of results. The majority of respondents were aged less than 30 years old (39%), residing in Australia (80%), and working within the acute care setting (38%; Table 10-1).

10.4.2 *Interest and perceived importance of dietary supplements*

When asked if they were interested in dietary supplements, the majority (65%) said that they agreed or strongly agreed. However, allied healthcare professionals were less decided when compared to the other respondents with 39% saying that they agreed or strongly agreed. When asked if dietary supplements were important to improving health outcomes, 49% agreed or strongly agreed while 35% said they were neutral.

10.4.3 *Perceived efficacy and safety of dietary supplements*

When asked if dietary supplements are safe, 60% stated that they were neutral with 17% and 22% saying they agreed and disagreed, respectively. When asked if they felt that dietary supplements are effective, similar trends were found with 61% stating they were neutral.

10.4.4 *Personal use of dietary supplements*

Respondents predominantly reported that they either never (34%) or occasionally (34%) consumed dietary supplements (Table 10-2). Doctors were the least likely to consume dietary supplements with 53% saying that they never consume dietary

supplement. Nurses were the next least likely profession to consume dietary supplements with 41% reporting never consuming dietary supplements. A small proportion (6%) of respondents also indicated that they sold dietary supplements as part of their clinical practice, these respondents were predominantly doctors.

10.4.5 *Sources and perceived access to information*

Fifty-five percent of respondents stated that they have access to reliable information regarding dietary supplements. When asked about where respondents access information regarding dietary supplements, over half of respondents listed the following sources: evidence-based databases, guidelines published by their professional body, academic journals, and their colleagues. When separated by profession, allied health professionals differed from this overall trend and instead reported the internet and their colleagues to be the most commonly reported sources of dietary supplement-related information. The majority of respondents said that in order for them to utilise a particular dietary supplement as part of their clinical practise, they required at least two to four favourable randomised controlled trials to be published.

10.4.6 *Adequacy of training*

In response to the statement “I was well trained in dietary supplements”, 58% said they either disagreed or strongly disagreed. The majority (80%) of respondents, particularly dietitians, indicated that they were interested in more training regarding dietary supplements and that universities should offer more training in this area (81%).

Table 10-2 Respondent attitudes, behaviours and use regarding dietary supplements

	Agree or Strongly Agree	Neutral	Disagree or Strongly Disagree
I am knowledgeable about dietary supplements.	56(187)	29(96)	15(50)
I am interested in dietary supplements.	65(216)	25(83)	10(34)
People in your profession are knowledgeable about dietary supplements	42(141)	35(116)	23(76)
I was well trained in dietary supplements.	16(53)	26(86)	58(194)
This area is important to improving health outcomes.	49(164)	34(113)	17(56)
Dietary supplements are effective.	35(115)	52(172)	14(46)
My profession should be knowledgeable about dietary supplements.	91(302)	6(20)	3(11)
My profession should be considered an authority on dietary supplements.	67(222)	18(60)	15(51)
There is a high demand for dietary supplements.	74(248)	20(66)	6(19)
I am often asked about dietary supplements by patients or clients.	79(264)	10(33)	11(36)
I feel confident in answering questions regarding dietary supplements.	47(155)	29(98)	24(80)
I am interested in further training on dietary supplements.	79(263)	14(46)	7(24)
Dietary supplements are safe.	17(57)	60(200)	23(76)
My profession should play a greater role in the prescription of dietary supplements.	58(193)	23(77)	19(63)
My profession should play a greater role in the education regarding the use of dietary supplements.	85(282)	9(30)	6(21)
My profession should play a greater role in research regarding the use of dietary supplements.	83(276)	11(37)	3(20)
I think universities should offer more training in these areas as part of their curriculum.	80(266)	13(43)	7(24)
I am able to access trustworthy information regarding dietary supplements.	56(185)	26(88)	18(60)
I regularly recommend dietary supplements to clients/patients.	25(84)	24(81)	50(168)

All values presented as row%(n)

In response to the statement “I am knowledgeable about dietary supplements”, 56% said they either agreed or strongly agreed. Approximately half (47%) of respondents felt that they were confident in answering questions about supplements. However, allied health professionals were the least likely to report that they were confident or knowledgeable about dietary supplements. Respondents cited a wide variety of areas in which they would like to improve their knowledge.

These included drug-supplement interactions and adverse effects of dietary supplements, reliable sources of information regarding dietary supplements, and the usage of dietary supplements for specific diseases (e.g. cancer) or goals (e.g. sports performance).

10.4.7 *Perceived barriers for use*

Respondents listed a wide selection of barriers to recommending supplements (Table 10-3). Concerns regarding potential interactions with other treatments (67%) was the most commonly indicated barrier for use. However, when responses were categorised by profession, there was a wide variation in the ranking of concerns.

10.4.8 *Perceived public and organisational opinions*

The majority of respondents believed that their viewpoints regarding dietary supplements would be similar to the viewpoints of dietitians (80%), their professional governing body (70%), and doctors (55%). The groups they believed were least likely to agree with their position were naturopaths (62%), followed by the general public (30%). When separated by profession, respondents indicated that members of their own profession would agree with their viewpoint; however, doctors were less decided as to

whether their governing professional body would agree (39% responded as agreed compared to 70% total sample). A greater number of nurses also indicated that naturopaths would agree with their viewpoints (68% compared to 18% of total sample)

Table 10-3 Perceived Barriers for use of dietary supplements by respondents

Barriers	%(n)
Concerns regarding potential interactions with other treatments	68(208)
A lack of training in this area	59(180)
Concerns about the regulation of dietary supplements	54(166)
Concerns regarding potential negative effects of dietary supplements	49(150)
Perceived lack of efficacy of dietary supplements	49(148)
A lack of confidence in this area	48(145)
Lack of authority to recommend dietary supplements to patients/clients	38(117)
Concerns regarding financial burden on patient	35(108)
It may conflict with the advice of other members from the patients/clients medical team	23(70)
Perceived Lack of quality dietary supplements on the market	22(67)
A lack of interest in this area	7(21)
Other	3(10)
No barriers, I recommend the use of dietary supplements.	3(8)

Respondents believed that the general public should primarily source dietary supplement-related information from dietitians (93%), doctors (76%), and pharmacists

(71%). However, close to half of nurses (44%) and allied health professionals (55%) indicated that naturopaths should also be a primary source of information. When respondents were asked who they believed the general public currently considers their primary source for information regarding dietary supplements, the most common responses were naturopaths (78%), the internet (73%), friends and family (65%), television and radio (64%).

10.5 Discussion

This study explored the attitudes, beliefs and behaviours of healthcare professionals in regards to issues related to dietary supplements. The results demonstrate that healthcare professionals are interested in dietary supplements; however, due to the large number of barriers health care professionals are tentative about integrating dietary supplements into their clinical practice.

Due to the potential concerns regarding the safety and contraindications that come with dietary supplement use, there is a need to ensure that the public is able and willing to access reliable information on this topic. Respondents believe that the general public do not prioritize healthcare professionals as their primary source of information regarding dietary supplements. Surveys that have specifically surveyed the general public's primary sources of dietary supplement-related information have reported healthcare professionals to be one of the common sources of information.[14,15] However, large use of other potentially less reliable sources such as the internet and magazines have also been reported.[14] Previous studies that have investigated the potential reasons for the public seeking other sources for dietary supplement-related information have found that patients

generally feel that their physicians are unsupportive of dietary supplement use or that a conversation about dietary supplements does not occur during consultation.[16]

Our survey found that only half (47%) of respondents considered themselves to be confident in this area, a figure that has been reported in similar surveys. For example, a previous survey of healthcare professionals also found that healthcare professionals were moderately confident in answering a set of questions regarding dietary supplements.[17] This level of confidence might be related to the large proportion of respondents that stated that tertiary training in this area is lacking (81%) and the high level of interest in further education (80%). This is supported by previous studies which reported similarly high levels of interest in further training.[18,19] For example, in a study of 162 dietitians, Lee et al.[19] reported that 75% of respondents were interested in further training. The introduction of evidence-based training to university curriculum would provide a reputable and widely-accessible avenue for reliable information regarding dietary supplements and would inform healthcare professionals regarding effective and responsible use of dietary supplements.

Concerns regarding potential interactions with other treatments was reported as the number one barrier (68%) to utilizing dietary supplements as part of clinical practice. From a perusal of the evidence base for various supplements, it is understandable how this may pose a significant concern. Many dietary supplements have potential safety concerns that have been identified through *in vitro* or animal studies but few have adequate clinical data that has explored the real-world impact of these concerns. A

pertinent example of this is the data regarding the potential anticoagulant effect of ginger consumption. *In vitro* data has consistently shown this to be a possible effect but clinical data has been inconsistent and has suffered from numerous limitations.[20] An additional limitation in the current literature is that the majority of studies on dietary supplements have been focused on the efficacy of the intervention while safety data has not been as thoroughly investigated. Furthermore, a possibly related finding of the survey was that 70% of respondents stated they would like to learn more about reliable sources of information regarding dietary supplements. A previous survey of military physicians found similar results with 65% stating that they did not feel they had reliable sources of information in this area.[21] There are a number of evidence-based databases that are aimed at informing clinicians about the effects of dietary supplements, promotion of these resources (e.g. through tertiary courses) would provide an easily-accessible source of information that would aid in addressing this barrier.

The results of this survey suggest that future interventions are required in order to evaluate the adequacy of current training regarding dietary supplements and investigate ways of improving education that is targeted towards healthcare professionals. In addition, future studies should explore the reasons that individuals access particular information sources over healthcare professionals so that approaches can be designed to address this.

We would like to acknowledge the following limitations of this study. First, a large proportion of respondents were dietitians compared to other healthcare

professionals. While the separation of results by profession was able to partially mitigate the overrepresentation of dietitians, the smaller cohort of non-dietitian healthcare professionals might have reduced the generalisability of the results. Second, the term "dietary supplements" encompasses a wide range of compounds and formulations, each with their own evidence base, safety and efficacy profile. It is conceivable that participant's responses may have been influenced by the restriction of questions to this definition as opposed to particular types of dietary supplements.

10.6 Conclusion

In summary, healthcare professionals are needed to effectively manage the widespread use of supplements by the general public. This survey study investigated the attitudes, beliefs, and behaviours of healthcare professionals and identified multiple barriers, implications for practice, and areas of future research. Primarily, future studies should evaluate current training approaches and to investigate ways of improving training and education that is targeted towards healthcare professionals. In addition, strategies to improve the confidence of healthcare professionals regarding this area should also be investigated.

10.7 References

1. Armstrong AR, Thiébau S, Brown LJ, Nepal B. Australian adults use complementary and alternative medicine in the treatment of chronic illness: a national study. *ustralian and New Zealand Journal of Public Health.* 2011;35:384–390.
2. Timbo BB, Ross MP, McCarthy PV, Lin CT. Dietary supplements in a national survey: Prevalence of use and reports of adverse events. *J Am Diet Assoc.* 2006;106(12):1966-1974.
3. Shalansky S, Lynd L, Richardson K, Ingaszewski A, Kerr C. Risk of warfarin-related bleeding events and supratherapeutic international normalized ratios associated with complementary and alternative medicine: a longitudinal analysis. *Pharmacotherapy.* 2007;27(9):1237-1247.
4. Molinari M, Watt KD, Kruszyna T, et al. Acute liver failure induced by green tea extracts: case report and review of the literature. *Liver Transpl.* 2006;12(12):1892-1895.
5. Momcilovic B. A case report of acute human molybdenum toxicity from a dietary molybdenum supplement--a new member of the "Lucor metallicum" family. *Arh Hig Rada Toksikol.* 1999;50(3):289-297.
6. Goodman GE, Thornquist MD, Balmes J, et al. The Beta-Carotene and Retinol Efficacy Trial: incidence of lung cancer and cardiovascular disease mortality

during 6-year follow-up after stopping beta-carotene and retinol supplements. *J Natl Cancer Inst.* 2004;96(23):1743-1750.

7. Gardiner P, Sadikova E, Filippelli AC, White LF, Jack BW. Medical reconciliation of dietary supplements: don't ask, don't tell. *Patient Educ Couns.* 2015;98(4):512-517.
8. Mitka M. Emerging data continue to find lack of benefit for vitamin-mineral supplement use. *JAMA.* 2014;311(5):454-455.
9. Wang C, Harris WS, Chung M, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr.* 2006;84(1):5-17.
10. Ried K, Frank OR, Stocks NP, Fakler P, Sullivan T. Effect of garlic on blood pressure: a systematic review and meta-analysis. *BMC Cardiovasc Disord.* 2008;8:13.
11. Heyland DK, Novak F, Drover JW, Jain M, Su X, Suchner U. Should immunonutrition become routine in critically ill patients?: A systematic review of the evidence. *JAMA.* 2001;286(8):944-953.
12. Hempel S, Newberry SJ, Maher AR, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: A systematic review and meta-analysis. *JAMA.* 2012;307(18):1959-1969.

13. Viljoen E, Visser J, Koen N, Musekiwa A. A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting. *Nutr J.* 2014;13:20.

14. Rozga MR, Stern JS, Stanhope K, Havel PJ, Kazaks AG. Dietary supplement users vary in attitudes and sources of dietary supplement information in East and West geographic regions: a cross-sectional study. *BMC Complement Altern Med.* 2013;13:200.

15. Ferrucci LM, McCorkle R, Smith T, Stein KD, Cartmel B. Factors related to the use of dietary supplements by cancer survivors. *J Altern Complement Med.* 2009;15(6):673-680.

16. Ben-Arye E, Polliack A, Schiff E, Tadmor T, Samuels N. Advising patients on the use of non-herbal nutritional supplements during cancer therapy: a need for doctor-patient communication. *J Pain Symptom Manage.* 2013;46(6):887-896.

17. Kemper KJ, Gardiner P, Gobble J, Woods C. Expertise about herbs and dietary supplements among diverse health professionals. *BMC Complement Altern Med.* 2006;6:15.

18. Box S, Creswell B, Hagan DW. Alternative Health Care Education in Dietetic Training Programs: A Survey of Perceived Needs. *Journal of the American Dietetic Association.* 2001;101(1):108-110.

19. Lee YK, Georgiou C, Raab C. The knowledge, attitudes, and practices of dietitians licensed in Oregon regarding functional foods, nutrient supplements, and herbs as complementary medicine. *J Am Diet Assoc.* 2000;100(5):543-548.

20. Marx W, McKavanagh D, McCarthy AL, et al. The Effect of Ginger (Zingiber officinale) on Platelet Aggregation: A Systematic Literature Review. *PLoS ONE.* 2015;10(10):e0141119.

21. Cellini M, Attipoe S, Seales P, et al. Dietary supplements: physician knowledge and adverse event reporting. *Medicine and science in sports and exercise.* 2013;45(1):23-28.

Part Three: Discussion and future directions

The aims of this thesis were to investigate the efficacy, safety, and feasibility of adjuvant ginger supplementation for CINV. In Part Three, the major results of the included studies are presented in relation to the study aims and hypotheses (Chapter 11), their methodological limitations and strengths of the individual studies are also discussed (Chapter 12), and finally, the implications for clinical practice and future research studies are presented in Chapter 13.

Chapter 11. Study results in relation to thesis aims and outcomes

The primary research question driving this PhD program described in this thesis was:

What is the efficacy, safety, and feasibility of ginger as an adjuvant treatment for chemotherapy-induced nausea and vomiting in chemotherapy-naïve patients undergoing highly- and moderately- emetogenic chemotherapy?

In this chapter, the major findings of each study are discussed in relation to the outcomes and hypotheses stated in the introduction (page 1).

Aim: To determine the *efficacy* of ginger as an adjuvant treatment for CINV

Outcomes:

- *To describe the mechanisms of action by which ginger could improve chemotherapy-induced nausea and vomiting.*

  In Chapter 4, the various mechanisms by which ginger could modify CINV were discussed. Before the publication of this manuscript, there had been no review of the potential mechanisms of action of the active constituents of ginger with respect to CINV. This review identified that the effect of ginger on the $5HT_3$ receptor was a likely mechanism that required further investigation.

In Chapter 6, the binding affinities of the principle ginger compounds within two binding sites on the murine 5-HT_3 receptor were investigated in order to reveal a preference for allosteric modulation.

The results demonstrated that that investigated ginger compounds had a high affinity to both binding sites and shared common residues with other known competitive antagonists including the setron class of compounds. These results provide further evidence that ginger compounds could act as 5-HT_3 antagonists.

- *To determine the optimal form of ginger to be used as an adjuvant therapy in clinical trials*

This outcome was investigated in Chapter 7, where HPLC analysis determined the forms of ginger that contained therapeutic doses of bioactive compounds. Per gram, ginger supplements, particularly the standardized extracts, were found to contain the greatest concentration of measured compounds (Mean±SD: 2.597±1.380 mg), while the concentration of compounds within spices (Mean±SD: 1.858± 1.346 mg), beverages (Mean±SD: 0.317± 0.210 mg), confectionary (Mean±SD: 0.093± 0.071 mg), and teas (Mean±SD: 0.025± 0.0002 mg) was considerably lower. Hence, standardized ginger extracts were determined to be the most suitable form of ginger for adjuvant use, due to the high content of bioactive compounds within the analysed products.

- *To determine the effect of ginger on i) CINV-related QoL and ii) the incidence, frequency and severity of chemotherapy-induced nausea and vomiting in chemotherapy-naïve patients receiving moderately or highly emetogenic chemotherapy regimens.*
    - *H_1: The standardized ginger extract will provide a significant reduction in measures of CINV-related QoL in patients receiving moderately or highly emetogenic chemotherapy regimens compared to placebo.*

  During the first chemotherapy cycle of the randomized controlled trial undertaken for this thesis (Chapters 8 and 9), the addition of a standardized ginger extract to standard anti-emetic medications was associated with a statistically significant improvement in nausea-related QoL (median [25^{th}, 95^{th} percentile]: 61.5 [56.1, 63] vs 54 [46, 63]; p=0.029) and CINV-related QoL (median [25^{th}, 95^{th} percentile]: 124.5 [113, 126] vs 111 [99.2, 126]; p= 0.043) when compared to placebo. These results suggest that the improvement in QoL was driven by an improvement in nausea-related QoL. However, these significant results did not continue during cycle 2 and 3. Possible explanations for this include: 1) a reduction in statistical power due to the number of dropouts during cycle 2 and 3; and 2) participants could have experienced a "response shift" in their perception of QoL (*see Chapter 9 discussion*). Therefore, while the results reported in Cycle 1 are sufficient to accept hypothesis H_1, this outcome requires further exploration in larger sample sizes.

- *H_2: The standardized ginger extract will provide a significant reduction in measures of acute chemotherapy-induced nausea in patients receiving moderately or highly emetogenic chemotherapy regimens compared to placebo.*

Previous studies in this field have reported significant reductions in acute nausea in patients who received ginger supplementation. It was therefore expected that ginger supplementation would provide the same significant benefit to patients in this study. However, no clinically or statistically significant difference in any measure of nausea was detected. Potential reasons for this difference include the generally low severity of symptoms reported by participants, which could indicate that the study was insufficiently powered to detect such differences. Because of these factors, the efficacy of ginger supplementation to reduce acute chemotherapy-induced nausea could not be adequately answered and hypothesis H_2 could neither be confirmed or rejected.

Aim: To determine the *safety* of ginger as an adjuvant treatment for CINV

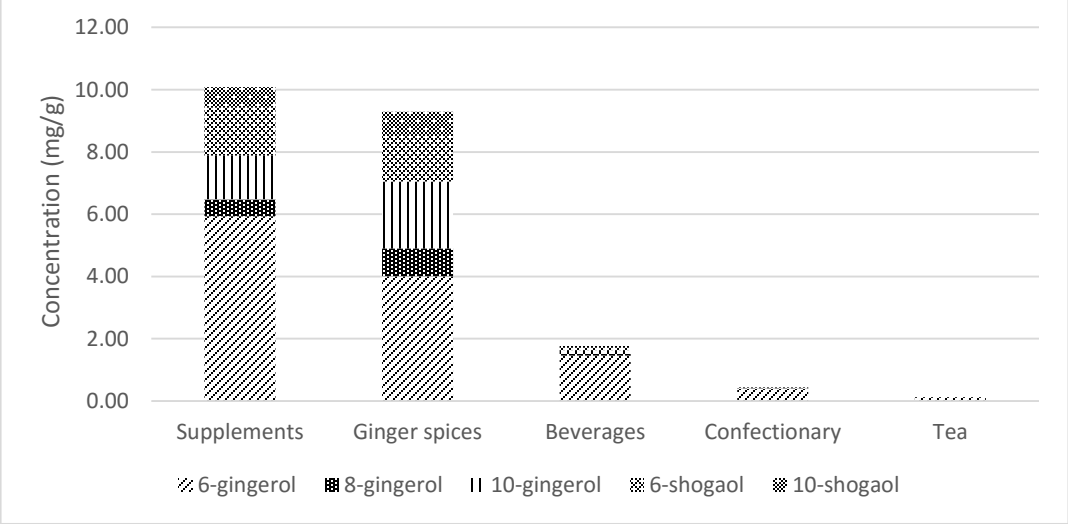
Outcomes:

- *To determine the dose of bioactive ginger compounds within a variety of ginger products*

As illustrated in Figure 11-1, there is considerable variation in bioactive compounds within the different categories of ginger products analysed in Chapter 7. When ginger

products were analysed in terms of the approximate concentration that would be consumed in one recommended serve of each product, there were dietary supplements as well as some confectionary and beverage products that contained large concentrations of the analysed compounds. Although the smallest effective dose of ginger is not yet elucidated, these results demonstrate that it is feasible to achieve an intake of the principle active compounds of ginger by consuming certain commercially available products that is comparable to the majority of dietary supplements analyzed in this study and therefore, ginger intake from food products should be controlled in future clinical trials.

Figure 11-1 Average concentration of analysed gingerol and shogaol compounds within each product category



- *To assess the safety profile of ginger in a clinical setting, including adverse effects and contraindications.*

  In the clinical trial (Chapter 9), ginger supplementation was well-tolerated with no significant increase in adverse events and few reported side-effects. This is consistent

with previous studies, which have reported minor adverse events.[8] Another potential concern is that ginger might reduce the effectiveness of anti-emetic therapy when patients are prescribed aprepitant. In the included study, participants receiving ginger supplementation in combination with aprepitant reported worse control of delayed CINV; however, this difference was not statistically significant. This relationship should be investigated in larger trials to ensure that patients' anti-emetic control is not compromised by ginger supplementation.

Aim: To determine the *feasibility* of ginger as an adjuvant treatment for CINV

Outcomes:

- *To determine the perceived confidence, reported use, and barriers for the use of dietary supplements such as ginger in clinical practice.*

  In Chapter 10's survey study, the attitudes, barriers and beliefs of 370 healthcare professionals were explored. Respondents were mixed in their rating of confidence. Approximately half (47%) of respondents felt that they were confident in answering questions about supplements but they considered their lack of confidence a barrier to using dietary supplements as part of clinical practice.

  Half of the respondents stated that they did not regularly recommend dietary supplements (25% agreed, 25% neutral). When asked if there were any enablers to recommending the use of dietary supplements, the most common response was "No enablers, I do not currently recommend the use of dietary supplements."

The results of the survey indicate that there are numerous barriers that prevent healthcare professionals recommending dietary supplements as part of clinical practice. More than half of the respondents expressed numerous barriers. Concern regarding potential interactions with other treatments was the number one reported barrier (67%). Healthcare professionals were also undecided regarding the efficacy and safety of dietary supplements. When asked if they agree with the statement "Dietary supplements are safe", 60% responded as neutral. Similarly, when asked if they agree with the statement "Dietary supplements are effective", 52% stated that they were neutral (35% agreed and 14% disagreed). In addition, approximately 50% or more of the respondents expressed concerns regarding insufficient training, regulation, adverse effects, and lack of confidence.

- *To assess the adherence of a standardized ginger regimen in a clinical setting.*

  Adherence to the study dosing regimen within the included clinical trial was moderate-to-high, with 70% of all participants (69% in ginger group and 75% in placebo group) consuming at least 3 of the 4 prescribed capsules per day. The results suggest that patients are generally able to adhere to the regimen included in this study. However, due to the attrition rate, it is possible that this dosing regimen, in combination with the questionnaires, could have contributed the perceived study burden.

Chapter 12. Limitations and strengths of studies undertaken during this research program

12.1 *In silico* investigation of principle ginger compounds on 5-HT$_3$ receptor binding

Using *in silico* modelling techniques, the potential binding affinities of the primary ginger compounds was investigated using the X-ray crystal structure of the murine 5-HT$_3$ receptor released in 2014[19]. Previous work in this area was restricted to homology models created from crystal structure of other CYS loop receptors. This study is the first *in silico* investigation to investigate the interactions between ginger compounds and the 5-HT$_3$ receptor and therefore, provides new information which can be used to further elucidate the potential mechanisms of action of ginger. Furthermore, this is the first *in silico* analysis investigating the binding affinities of several other competitive antagonists (such as serotonin and ondansetron) using the crystal structure of a 5-HT$_3$ receptor. By contrasting the orientations and theoretical binding affinities of the ginger ligands with known agonists, competitive and non-competitive antagonists known to interact with the 5-HT$_3$ receptor as well as decoys compounds, this study was able to provide further support for the action of these compounds as modulators of 5-HT$_3$ receptor activity. Furthermore, we were able to identify potentially key binding residues at both the serotonin and allosteric sites and corroborate with other research the importance of previously identified as residues important for binding serotonin and other competitive antagonists.

While this study provided valuable information regarding the binding affinities of the primary ginger compounds with the 5-HT_3 receptor as well as several other competitive antagonists, there exist some limitations from a theoretical viewpoint which may impact on the results obtained. For example, the 3.5Å resolution of the X-ray structure used in the analysis is relatively low making it difficult for the crystallographer to unambiguously assign atomic coordinates. This level correlates to a resolution where the backbone atom positions and those of the bulky side chains are mostly visible. A higher resolution of around 1.2 Å would have provided more accurate positioning. which means the impact of this limitation was somewhat reduced by conducting energy minimisations of the receptor to reduce local areas of strain. Additionally, Hassain et al.[19] speculated that the crystal structure that was captured is in the closed conformation, meaning that the ligands could act differently within the selected binding sites if the crystal structure was in a different conformation.

Molecular docking relies on classical molecular mechanics to estimate binding energies. Compared to techniques which incorporate quantum mechanics to explore the energetics of molecular interactions, classical mechanics is not as accurate however computations for large atomic systems using quantum mechanics is not feasible at the current time. Molecular docking techniques which incorporate flexibility in the target residues lining the ligand binding site offer a more realistic approach to estimating the binding affinities of protein-ligand interactions. Our analysis used a more rigid approach to the docking operation and could limit the degree to which the conformational space could be explored by the ligands. To date, no 5-HT_3 crystal structures exists with a ligand

bound to either the serotonin binding or the allosteric site 5-HT_3 receptor; however, if published in the future, this will be an area of further investigation.

12.2 The concentration of major active constituents within commercial ginger products using reverse phase-high performance liquid chromatography

In this study, the concentration of principle bioactive compounds within 20 commercial ginger products were quantified. To achieve this, a protocol was developed using reverse phase HPLC analysis, which is a widely-used and validated method that delivers a high level of precision. It is also superior to other methods such as gas chromatography–mass spectrometry, which, due to the increase in temperature, can result in a degradation of compounds and therefore affect the results of the analysis.[20]

This study also expanded on the current literature by including the following additions to the study protocol. First, we have expanded on previous studies by including an additional compound, [10]-shogaol, in the analysis. [10]-shogaol has not been extensively studied; however, *in vitro* research suggests that it possesses anti-inflammatory properties and might aid wound healing[21,22] This compound has been rarely quantified in commercial products so by including it in this analysis, this study provided information regarding the concentration of this compound in a large variety of previously unanalysed commercial ginger products.

The range of products analysed was also expanded, which aids current understanding of the typical concentration of compounds within different types of ginger products. The range of products used in this study is of particular benefit to Australian

practitioners and consumers, as all products are readily available in Australian stores. Furthermore, as part of the study protocol, the yield of the two extraction procedures used in this study was validated using a mix of ginger standards of a predetermined quantity. This process improves the accuracy of the analysis and is a significant strength as this procedure was not undertaken consistently in previous studies.

While this study was able to determine the concentration of several of these principle compounds, numerous additional compounds could not be analysed due to the lack of commercial standards for these compounds. In addition, the samples used in this study were purchased locally (as opposed to purchasing directly from the manufacturer). The concentration of compounds could therefore have been influenced by factors such as storage conditions, which might make them unrepresentative of the initial products. However, this does provide valuable insight into the concentration of these compounds at time of purchase to the consumer, which reflects the "real world" concentration of compounds in these products as received by consumers.

12.3 The effect of a standardized ginger extract on chemotherapy-induced nausea and vomiting related quality of life in patients undergoing moderately and highly emetogenic chemotherapy: a randomized controlled trial.

The clinical trial undertaken as part of this PhD thesis addressed several key limitations in the current research literature (*see Chapters 2 and 3*). It also investigated previously unexplored outcomes such as nutrition status and cancer-related fatigue (*see Chapters 8 and 9*).

The Academy of Nutrition and Dietetics published an Evidence Analysis Manual designed to guide the systematic appraisal of the literature.[23] Using the Quality Criteria Checklist provided as part of this manual, studies are assigned a positive, neutral or negative quality rating based on the studies inclusion of measures that aim to reduce bias and confounding factors. Using this checklist (Appendix G), the trial presented in Chapters 8 and 9 meets the required criteria to the point where it can be classified as a 'positive' quality study, incorporating several features that represent robust, gold standard study design and methodology. These include double blinding of investigators and patients to the allocation procedure, the inclusion of a placebo study arm and randomised allocation of participants to the intervention of placebo group. In addition, using the NHMRC National Health and Medical Research Council (NHMRC) Hierarchy of Evidence Guidelines (IV-I, with I being the strongest level of evidence), this trial provides level II evidence.

This study further included robust study design features that are specific to the investigation of interventions to treat CINV. Parallel and cross-over styles of RCT study design are two viable study designs that were employed in previous clinical trials that investigated ginger supplementation. Both have advantages and disadvantages; however, when conducting research relating to the study of anti-emetic interventions, there are unique considerations that warrant special consideration. Cross-over trials offer many advantages, including the ability to avoid inter-patient variability, and are of particular interest to studies with limited resources or time constraints because they reduce the sample size needed for parallel study designs.[24] However, the advantages of a cross-over

trial do not outweigh their risks in anti-emetic studies for several reasons. First, there is considerable variation in CINV experiences between cycles and therefore, by following this study design, there is an increased risk of within-patient variability, a variation that could negate the reduction in inter-patient differences.[25] Additionally, due to the impact that anti-emetic control during the initial cycle of chemotherapy has on CINV control during subsequent cycles, the cross-over design is problematic as the initial intervention will significantly influence the results of the subsequent intervention when the study is crossed over.[26] Ethical considerations also come into play if anti-emetic medications are changed when the initial intervention is effective. A parallel study design is clearly preferable when studying anti-emetic interventions and was the approach used in this study.

Another strength of this study was that it was prospective and pragmatic, studying the range of patients encountered in clinical practice. This enhances the applicability of the results to a significant proportion of the cancer population. Randomised controlled trials in homogenous populations, while considered a gold standard study method, have at times been criticized for their reduced external validity, which is caused by the constraints of the study design (e.g. narrow inclusion/exclusion criteria) leading to results with limited generalisability.[27] This study followed patients over three chemotherapy cycles in order to obtain a more accurate assessment of their experience with CINV over the course of their chemotherapy (which tends to worsen with time) instead of capturing outcomes in one isolated cycle, as was done in most previous studies.[8] Patients were also enrolled on the basis of the emetogenicity of their chemotherapy regimen, reflecting the

wide range of regimens encountered in clinical practice, rather than confining the sample to specific regimens (e.g. cisplatin-based regimens only[28,29]) or specific cancer types (e.g. breast cancer patients only[30]).

This study is also the first to investigate the effect of ginger supplementation on fatigue using a validated questionnaire. Cancer-related fatigue is a highly prevalent and burdensome symptom for cancer patients.[31] Reasons for the observed improvement in fatigue in this study are currently unclear. A possible explanation is that nausea and vomiting can result in fatigue due to low food intake as well as affecting quality of life. However, the significant association between the ginger intervention and reduced fatigue at cycle 3, despite low levels of nausea, suggests that there could be an additional mechanism of action. The effect of ginger on cognition, mood, or fatigue was not rigorously assessed in previous studies and should be further explored in future studies.

An additional strength of this study was the analysis of the intervention and placebo capsules at the commencement and completion of the clinical trial using HPLC analysis in order to determine the potency of the intervention over the trial period. This is a significant strength because ginger, as with all herbal formulations, contains a large variety of bioactive compounds that can exert various effects on the human body. By ensuring the formulation contained a therapeutic dose of the active compounds throughout the trial period, it can be concluded that sufficient concentrations of the bioactive compounds were present at all times and that there was no significant degradation of the intervention over time.

The following potential limitations of the study are also acknowledged. The attrition rate is a primary limitation of this clinical trial. This could be attributed to the harsh nature of chemotherapy as well as the potentially burdensome study design of the clinical trial. Due to the number of withdrawals in cycles 2 and 3, it is likely that this resulted in under-powering of the study and subsequent inability to detect significant differences in CINV-related measures at these specified time points. Future studies should include inflation factors in their sample size calculations to mitigate attrition and implement strategies to reduce dropouts. In addition, a questionnaire was developed for this study to assess the presence of prognostic factors that are understood to influence the risk of CINV. Unfortunately, due to the low completion rate of this questionnaire, these prognostic factors could not be analysed. However, prognostic factors including chemotherapy emetogenicity, gender, and age were recorded during the initial participant interview and so were able to be included in the analysis. Based on the observations of this trial, the following strategies could be implemented to prevent attrition in future studies in this area:

- The perceived burden of the study might be reduced by condensing the study questionnaire booklet. This could be achieved by reducing the number of investigated outcomes or by selecting alternative validated questionnaires that contain fewer items. For example, the Multinational Association of Supportive Care in Cancer (MASCC) Anti-emesis Tool is a validated questionnaire that assesses acute and delayed CINV by asking four questions at two separate time points (24 hours after chemotherapy and 4 days post-chemotherapy).[32]

- Similarly, the replacement of the study booklet with mobile apps could reduce the burden further as patients would no longer need to mail the booklet, it could appear more visually appealing, and some apps provide reminders that could help patients with adherence to the study protocol. The MASCC Anti-emesis Tool is available as an app that allows the patient to send their questionnaire responses to the investigators in real time. However, the digital literacy of potential participants would need to be assessed before enrolment to avoid participant confusion and improper use.
- More regular contact with participants could improve adherence. In our study, patients were contacted at the start of each chemotherapy cycle and five days post-chemotherapy via telephone. Providing text reminders or additional phone calls could help provide support to the participants.
- Due to the funding constraints of this study, we were unable to offer incentives (e.g. money, vouchers) to participants for their participation. Completion rates could be improved by providing tangible incentives.

12.4 The attitudes, beliefs and behaviours of healthcare professionals in regards to dietary supplements.

The results of this survey study provided a comprehensive assessment of the perceptions of healthcare professionals with respect to key issues related to dietary supplements. In total, 370 healthcare professionals responded, which is similar to, or larger than, previous studies in this area.[33,34] Methods used to contact a large sample of dietitians resulted in a strong response. Although significant efforts were made to increase

the number of respondents from other professions (e.g. pharmacists), these strategies were not successful. Consequently, relative to dietitians, only a small number of other healthcare professionals were recruited. The high proportion of dietitian respondents (78%), particularly Australian dietitians, is a significant contribution to the literature due to the lack of any previous studies in this population. However, this is also a limitation as it reduces the ability of the results to be extrapolated to the general healthcare community. Future studies should consider additional avenues of disseminating to various healthcare professionals in order to improve the generalisability of results.

This study specifically focused on dietary supplements rather than the more general term, "complementary and alternative medicines". This term, which embraces a variety of treatment modalities, was used in several previous studies.[35] However, the term "dietary supplement" also embraces several different preparations (e.g. herbal, vitamin, mineral and amino acid components) and could have been responsible for the high level of neutral responses on some questions. For example, when asked if respondents thought dietary supplements are safe, 60% responded as neutral. However, the primary aim of this survey was to determine how healthcare professionals view dietary supplements as whole and so the undecided responses to some questions provide meaningful information towards this aim.

Chapter 13. Overall implications for clinical practice and future research directions

Adjuvant ginger supplementation is a low-cost, widely available intervention that healthcare professionals could utilise in order to provide a significant benefit to cancer patients undergoing chemotherapy without significant side effects. Due to the growing clinical interest and treatment potential of ginger supplementation, this thesis aimed to improve the current understanding of the efficacy, safety, and feasibility of ginger supplementation as part of clinical practice. In this chapter, the implications of the research conducted in this thesis are discussed, along with recommendations for clinical practice and future research.

13.1 Assessment of the current body of evidence

In Chapters 2 and 3, the use of ginger supplementation was reported to be a promising intervention for CINV but one with insufficiently high-level evidence to demonstrate a clear effect. At the time of the initial systematic literature review, the evidence for the use of ginger as an adjuvant treatment for CINV was graded as 'C' according to the NHMRC evidence-based guidelines,[36] indicating that the evidence provided some support for the use adjuvant ginger supplementation in treating CINV but that clinical judgment was required due to existing limitations. Due to the continued research in this area, it is appropriate to reassess the evidence grade presented in our initial systematic review.

In order to create an evidence-based recommendation, the NHMRC guidelines recommend that the literature is assessed according to five separate criteria: the strength of the evidence, consistency, clinical impact, generalizability and applicability to the Australian context. The evidence-base for adjuvant ginger supplementation will now be discussed using these criteria.

Strength of the evidence: This criterion includes the number of studies, level of evidence and risk of bias in the included studies). Eight clinical trials are published in this area, in addition to the clinical trial presented in this thesis. However, many of these studies include some or all of the limitations cited in our original literature review (Chapters 2 and 3) and not all are double-blind, parallel (as opposed to crossover) studies.

Consistency of results: While the limitations described in Chapter 2 are still present in many of these studies, most studies have consistently reported adjuvant ginger supplementation to reduce measures of CINV. When combined with the studies included in our initial review, from a total of 9 studies (including the trial conducted as part of this thesis) 6 reported ginger supplements to be associated with significant improvements in CINV and 3 found no effect.[8,37,38]

Clinical impact: Although the clinical significance of ginger supplementation in our trial was minor, the majority of previous studies (all of which recruited patients with previous experience of CINV) have generally reported moderate reductions in measures of CINV.

Generalizability: The study design and pragmatic cohort used in most studies reflect common cancer populations, antiemetic medications and chemotherapy regimens. Because of this, the results of the majority of studies in this area are generalizable to the general cancer population with few caveats.

Applicability to the Australian context: The applicability of these studies to the Australian context is difficult to elucidate. As discussed in Chapter 9, the average severity of CINV and CINV-related QoL in our study was low. A potential explanation is that while the *prevalence* of CINV remains high, due to uptake of evidence-based anti-emetic guidelines in combination with the introduction of anti-emetics such as aprepitant, the average *severity* of CINV is low at the recruiting hospital used in this trial. If the severity of CINV identified in our trial is similar in other major Australian hospitals then this suggests that the addition of ginger supplementation to current antiemetic therapy is not necessarily useful in the Australian context. It could be more useful in cancer populations that do not have reliable access to current generation anti-emetics such as in developing countries. Indeed, the control of CINV in Australia is reported to be high, with one study reporting Australia to have the lowest prevalence of vomiting in patients receiving MEC when compared to five other Oceanic countries.[2]

Table 13-1 NHMRC Body of evidence matrix

Component	A	B	C	D
	Excellent	**Good**	**Satisfactory**	**Poor**
Evidence base[1]	several level I or II studies with low risk of bias	one or two level II studies with low risk of bias or a SR/multiple level III studies with low risk of bias	level III studies with low risk of bias, or level I or II studies with moderate risk of bias	level IV studies, or level I to III studies with high risk of bias
Consistency[2]	all studies consistent	most studies consistent and inconsistency may be explained	some inconsistency reflecting genuine uncertainty around clinical question	evidence is inconsistent
Clinical impact	very large	substantial	moderate	slight or restricted
Generalisability	population/s studied in body of evidence are the same as the target population for the guideline	population/s studied in the body of evidence are similar to the target population for the guideline	population/s studied in body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population[3]	population/s studied in body of evidence differ to target population and hard to judge whether it is sensible to generalise to target population
Applicability	directly applicable to Australian healthcare context	applicable to Australian healthcare context with few caveats	probably applicable to Australian healthcare context with some caveats	not applicable to Australian healthcare context

Using the NHMRC Body of evidence matrix (Table 13-1) to guide this recommendation, it is evident that there is *mostly consistent* evidence from *multiple level II studies that contain moderate risks of bias* to indicate that adjuvant ginger supplementation is associated with *moderate* reductions in CINV in *populations that are probably similar to that of the Australian cancer population* and that are *probably applicable to the Australian healthcare context with some caveats*. Hence, although there is continued research in this area, due to the mixed results and extant limitations, the recommendations from our previous review are still appropriate and therefore, there is C level evidence, indicating that the "Body of evidence can be trusted to guide practice in most situations" (Figure 13-1).

Figure 13-1 Definition of NHMRC grades recommendations

Grade of recommendation	Description
A	Body of evidence can be trusted to guide practice
B	Body of evidence can be trusted to guide practice in most situations
C	Body of evidence provides some support for recommendation(s) but care should be taken in its application
D	Body of evidence is weak and recommendation must be applied with caution

However, further studies are required to address additional clinically-relevant issues including the optimal dosing regimen and drug-nutrient interactions. Until these issues are addressed, the use of adjuvant ginger supplementation requires clinical judgment by the healthcare professional. Using the existing literature for reference, the patient should be informed of the optimal type, amount and frequency of dosage. Specifically, the current evidence suggests that the optimal dosing regimen is 0.5-1g of a standardized extract that is divided into four capsules per day and consumed approximately every 4 hours (e.g. 250mg with each meal) commencing on the day of chemotherapy and continuing for the following 5 days. In addition, the monitoring of adverse events (e.g. thrombocytopenia) and the management of drug-nutrient interactions (e.g. patients on anticoagulant therapy) needs to be observed.

13.2 Safety implications associated with ginger supplementation

Commonly prescribed medications are associated with a wide-range of known side-effects; however, this does not preclude their use in the clinical setting. There are well-explored safety profiles with approved medications. In addition, health care

professionals are well-trained in the appropriate use of these medications. In order to integrate dietary supplements into clinical practice, these interventions require a safety profile that is elucidated to the same extent as standard medications.

As demonstrated by the results of the systematic literature review on the potential anti-platelet effect of ginger (Chapter 5) as well as the results from the survey study, which found that concern regarding drug-nutrient interactions was the primary concern of healthcare professionals, it is evident that further research is required in order to investigate the safety profile of ginger supplementation in the clinical setting. While adverse events were monitored during our clinical trial, the safety profile of ginger should be further explored in clinical trials in order to inform healthcare professionals who are interested in the therapeutic use of ginger. The clinical trial undertaken as part of this thesis regularly assessed side-effects associated with the intervention through multiple methods, including a pre- and post- cycle safety assessment questionnaire during treatment, patient interview and perusal of patients' medical chart for documented adverse events. Future studies should expand on this by including the assessment of objective measures such as blood chemistry. In particular, the platelet count and measures of blood coagulation such as INR as well as close monitoring for physical indications of abnormal bleeding (e.g bruising) should be investigated to determine the effect of ginger on platelet aggregation during chemotherapy. If strategies such as these are implemented, the amount of data regarding the safety of dietary supplements will be improved.

Furthermore, the results of the HPLC study (Chapter 10) indicate that it is feasible for patients to achieve an intake of the principle active compounds of ginger by consuming certain commercially available products that is comparable to the majority of dietary supplements analysed in this study. These results will be of value to healthcare practitioners, particularly nurses, who regularly recommend ginger products to patients who experience nausea and vomiting, as well as to patients who could be seeking ginger for its potential effect against nausea. However, this also suggests that certain food products that contain large quantities of ginger could be able to exert a comparable effect on platelet aggregation when compared to dietary supplements. This also highlights the need for future clinical trials to monitor participant intake of ginger products as this could significantly affect the amount of total active compounds consumed by participants and therefore, confound results.

13.3 Dissemination of evidence-based recommendations

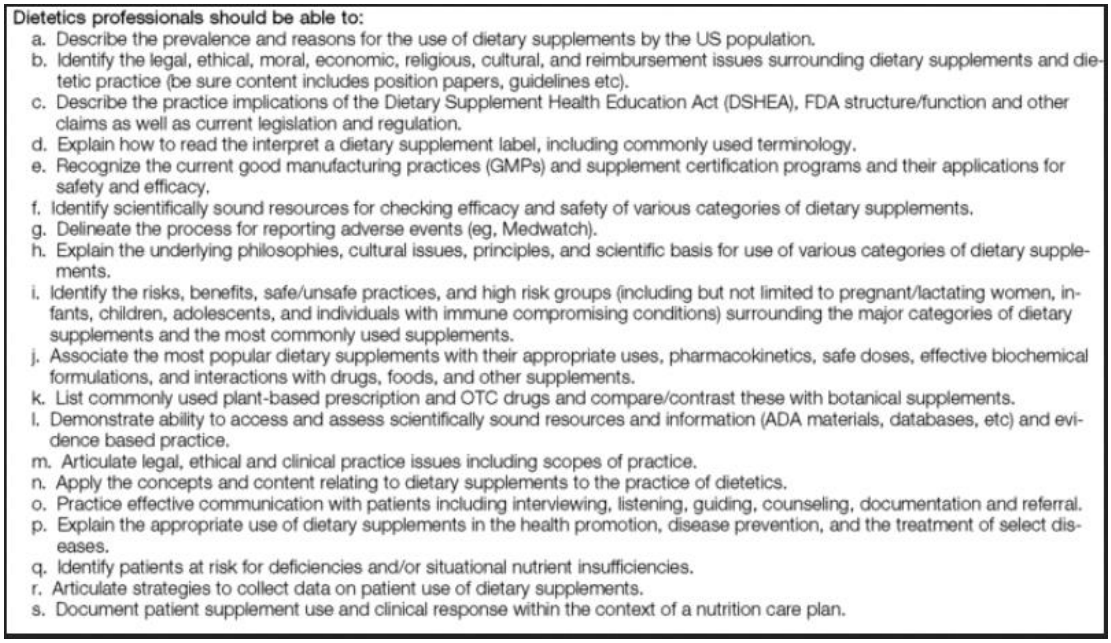
One of the aims of the research program presented in this thesis was to investigate the feasibility of ginger supplementation as an adjuvant treatment for chemotherapy-induced nausea and vomiting. An additional step that is required before dietary supplements are recommended for clinical practice is the dissemination of data regarding the safety and efficacy of dietary supplements such as ginger to healthcare professionals. The respondents of the included survey (Chapter 7) reported a significant lack of training (58% respondents indicated that they were not well-trained) and a strong interest in further training in dietary supplements (80%). The results of this study can inform current training approaches.

Many educational resources are available to assist healthcare professionals with dietary supplementation. These include short courses delivered by the Australasian College of Nutritional and Environmental Medicine, and many Australian universities offer postgraduate courses in complementary medicine including RMIT University (Master of Wellness), University of Tasmania (Graduate Certificate in Evidence-based Complementary Medicine), and the University of New England (Master of Health Science). However, the results of our survey suggest that such resources are underutilized. One possible explanation for this is that while many of these university courses include training in dietary supplements, they also include training in other complementary therapies that might not be relevant to all healthcare professionals. In addition, these are separate courses that a healthcare professional would need to complete in addition to their professional degree, entailing additional time and financial commitments. An alternative to this is the introduction of evidence-based training into existing university curricula for required professional degrees. A large majority (80%) of respondents from our study said that they think universities should provide training in this area as part of their professions curricula, which demonstrates a clear demand for this type of tertiary training. By integrating dietary supplement-related education into existing curricula, it would reduce the potential time and financial burden of additional course work and would also ensure that all graduates of professional degrees receive a consistent education. The problem remains, however, of how to do so in the context of already crowded curricula, the content of which is primarily driven by legislative and accreditation requirements.

Dietitians are in a key position to help educate patients and the general public about dietary supplements. Surveyed dietitians were highly interested in this area (68%). This interest can also be seen from the strong membership rates (>300 members) of the Dietitian's Association of Australia (DAA) Integrative Medicine interest group. However, respondent dietitians felt that they had not previously received adequate training in this area (55%). In the DAA National Competency Standards for Entry Level Dietitians, there are currently no standards that specifically address dietary supplements. Due to the strong interest in further training expressed by the practising dietitians in this survey, the addition of competency standards that directly relate to the education, research, and prescription of dietary supplements requires consideration.

Curriculum requirements for US dietetics education state that "graduates will have knowledge of complementary and alternative nutrition and herbal therapies" and that "graduates will have knowledge of dietary supplements".[39] Furthermore, a taskforce initiated by the Academy of Nutrition and Dietetics developed a set of competencies related specifically to dietary supplements (Figure 13-1) and while integration of these competencies into university curricula was inconsistent, many US universities now offer training in this area as part of the require dietetic degrees.[40,41] Hence, a possible example for how Australian dietetics could progress dietary supplement-related training is by mirroring the initiatives introduced in the USA.

Figure 13-2 Dietary supplement-related competencies



Dietetics professionals should be able to:

a. Describe the prevalence and reasons for the use of dietary supplements by the US population.
b. Identify the legal, ethical, moral, economic, religious, cultural, and reimbursement issues surrounding dietary supplements and dietetic practice (be sure content includes position papers, guidelines etc).
c. Describe the practice implications of the Dietary Supplement Health Education Act (DSHEA), FDA structure/function and other claims as well as current legislation and regulation.
d. Explain how to read the interpret a dietary supplement label, including commonly used terminology.
e. Recognize the current good manufacturing practices (GMPs) and supplement certification programs and their applications for safety and efficacy.
f. Identify scientifically sound resources for checking efficacy and safety of various categories of dietary supplements.
g. Delineate the process for reporting adverse events (eg, Medwatch).
h. Explain the underlying philosophies, cultural issues, principles, and scientific basis for use of various categories of dietary supplements.
i. Identify the risks, benefits, safe/unsafe practices, and high risk groups (including but not limited to pregnant/lactating women, infants, children, adolescents, and individuals with immune compromising conditions) surrounding the major categories of dietary supplements and the most commonly used supplements.
j. Associate the most popular dietary supplements with their appropriate uses, pharmacokinetics, safe doses, effective biochemical formulations, and interactions with drugs, foods, and other supplements.
k. List commonly used plant-based prescription and OTC drugs and compare/contrast these with botanical supplements.
l. Demonstrate ability to access and assess scientifically sound resources and information (ADA materials, databases, etc) and evidence based practice.
m. Articulate legal, ethical and clinical practice issues including scopes of practice.
n. Apply the concepts and content relating to dietary supplements to the practice of dietetics.
o. Practice effective communication with patients including interviewing, listening, guiding, counseling, documentation and referral.
p. Explain the appropriate use of dietary supplements in the health promotion, disease prevention, and the treatment of select diseases.
q. Identify patients at risk for deficiencies and/or situational nutrient insufficiencies.
r. Articulate strategies to collect data on patient use of dietary supplements.
s. Document patient supplement use and clinical response within the context of a nutrition care plan.

13.4 Additional future directions

13.4.1 *Investigation of ginger supplementation within unexplored cancer populations*

To date, trials that have investigated the use of ginger supplementation for CINV primarily focused on patients undergoing highly- and moderately- emetogenic chemotherapy regimens. The results of a clinical trial by Sontakke et al.[42] demonstrated that ginger supplementation was able to reduce CINV to the same extent as the antiemetic metoclopramide. While metoclopramide is no longer recommended as a first line therapy in highly- and moderately- emetogenic chemotherapy regimens, it is still used during low emetogenic chemotherapy regimens or as a PRN rescue anti-emetic. Due to the low-cost and safety profile of ginger, future clinical trials should investigate whether ginger supplementation could be an effective alternative to metoclopramide when used during low emetogenic chemotherapy regimens or as a rescue anti-emetic.

13.4.2 *Rigorous assessment of blinding*

While a *post-hoc* analysis was conducted as part of the clinical trial presented in this thesis to determine the efficacy of blinding measures, due to the distinct taste and aroma of ginger, it would be useful to assess the efficacy of the blinding procedure before the commencement of future clinical trials. This could be performed by randomly administering either the intervention or placebo to a small subset of cancer patients or healthy participants for a timeframe that resembles the timeframe of the intended study and recording the ability of participants to correctly guess their assigned group.

13.4.3 *Further research regarding dosing regimens and preparations*

In the clinical trial included in thesis, it was postulated that a daily dosing regimen of four capsules per day would provide superior protection when compared to the once or twice daily regimen that was used in previous studies. While we were able to find significant differences between the intervention and placebo when using this regimen, our trial design did not allow us to compare the efficacy to other dosing regimens. In future studies, it is recommended that the effect of different dosages is further explored. Due to the potential burden of the dosing regimen used in this study, it would also be of interest to explore the efficacy of slow-release capsule formulations, as this would potentially provide same consistent plasma concentration of ginger while reducing the required effort of the patient.

13.4.4 *Investigation of the pharmacokinetics, absorption, and bioavailability of different ginger products*

There is limited data on the absorption and excretion of ginger compounds in humans.[43,44] Further studies in this area would be of use to clinical trials as information from these studies could be used to inform optimal dosing regimens. In particular, as the mechanism of action of ginger is likely to be in the gut, future studies are needed to assess the pharmacokinetics of ginger compounds within the gastrointestinal tract. Furthermore, as demonstrated by the multiple ginger products analysed in Chapter 7, there are a number of different ginger food products and supplement preparations. Currently, the limited pharmacokinetic data available pertains to dietary supplements. It would be of interest to clinicians and patients to also determine the pharmacokinetics of active ginger compounds within different food products.

13.4.5 *Investigation of the effect of ginger on intercycle nausea and vomiting*

Currently, there is limited investigation related to the prevalence and management of intercycle CINV, nausea and vomiting that occurs 14-16 days after a chemotherapy cycle.[45] However, current evidence suggests that this can occur in up to 27% of patients.[45] Therefore, future studies could examine the effect of these symptoms on QoL as well as the effect of anti-emetic interventions such as ginger supplementation on the management of these symptoms,

13.4.6 *In silico investigation of principle ginger compounds within additional sites within the 5-HT$_3$ receptor*

Site-directed mutagenesis studies have to date identified a number of key residues important for binding serotonin or other competitive agonists/antagonists as well as other potential binding sites for allosteric modulation within the 5-HT$_3$ receptor. From this information we selected two sites of interest for comparison. Though the evidence to date suggests that the transmembrane channel could be a likely site for allosteric modulation it is more likely that ligands acting there are more lipophilic in nature than the ginger ligands. However, there may well be other potential sites that and future *in silico* studies could focus on other previously unexplored regions of the receptor. An important aspect to the action of these receptors is the stoichiometry of the subunits and its impact ligand-induced alterations in activity. Although this study focused on the homomeric A+A- receptor future work could compare the results obtained here with that of a heteromeric receptor, A+B- or B+A-, for example. While awaiting further crystal structures to become available, these heteromeric models could be created by homology modelling techniques.

13.4.7 *Further investigation into the effect of ginger on fatigue*

Fatigue is the most common side-effect reported by cancer patients, with up to three quarters of patients undergoing chemotherapy or radiotherapy reporting significant fatigue.[46] Several interventions have been investigated for their effect on fatigue, including dietary supplements such as carnitine. Ginger, however, has not been previously investigated as an intervention for fatigue but due to the results of the clinical

trial included in this thesis, further trials are recommended to confirm these results and if replicated, to investigate the potential mechanism by which it exerts this effect.

13.4.8 *Expanding HPLC analyses to multiple batches*

One of the limitations of the HPLC analysis included in this thesis (Chapter 10), is that only one sample of each product was analysed. Due to the influence of heat, moisture, length of storage, and origin of ginger on the analysed compounds, future studies should compare the concentration of compounds in multiple batches of the analysed product in order to determine a more representative quantification of the compounds within a particular product.

Chapter 14. Conclusion

Adjuvant ginger supplementation for the treatment of nausea is an example of a widely-used dietary supplement with promising evidence to support its use. In a series of reviews and studies, the research program presented in this thesis investigated adjuvant ginger supplementation for the treatment of CINV, the level of active compounds within various commercial ginger products, and explored the mechanisms by which ginger could interact with key pathways involved in CINV. The results of the main study in this thesis, the randomized controlled trial addressed multiple previous limitations in the literature and in doing so, demonstrated ginger supplementation to be significantly associated with improved CINV-related QoL and cancer-related fatigue. However, no significant reduction in the prevalence and severity of CINV were reported. The results of the HPLC analysis demonstrated that dietary supplements as well as certain ginger-based confectionary and beverages contained sufficient quantities of active compounds to be potentially protective against nausea. In addition, the survey results presented in this thesis indicate that there a number of barriers to the effective use of dietary supplements such as ginger extract by healthcare professionals. In particular, concerns regarding drug-nutrient interactions and insufficient training were primary barriers identified.

Ginger supplementation is a low-cost, widely-available, well-tolerated and potentially effective adjuvant treatment for CINV. While this thesis provides evidence for the use of adjuvant ginger supplementation for CINV-related QoL, future studies are needed to elucidate the efficacy in reducing the prevalence of CINV and should focus on

the safety and optimal dosage of ginger supplementation in patients undergoing chemotherapy treatment. Furthermore, the dissemination of evidence-based information and the further integration of education regarding dietary supplements into tertiary training is recommended to inform healthcare professionals and clinical practice. If larger studies address these recommendations, the use of adjuvant ginger supplementation will be a viable adjuvant treatment that healthcare professionals could utilise in order to improve CINV-related outcomes in clinical practice.

References

1. Olver IN, Eliott JA, Koczwara B. A qualitative study investigating chemotherapy-induced nausea as a symptom cluster. *Support Care Cancer.* 2014;22(10):2749-2756.
2. Hsieh RK, Chan A, Kim HK, et al. Baseline patient characteristics, incidence of CINV, and physician perception of CINV incidence following moderately and highly emetogenic chemotherapy in Asia Pacific countries. *Support Care Cancer.* 2015;23(1):263-272.
3. Sun CC, Bodurka DC, Weaver CB, et al. Rankings and symptom assessments of side effects from chemotherapy: insights from experienced patients with ovarian cancer. *Support Care Cancer.* 2005;13(4):219-227.
4. Bloechl-Daum B, Deuson RR, Mavros P, Hansen M, Herrstedt J. Delayed nausea and vomiting continue to reduce patients' quality of life after highly and moderately emetogenic chemotherapy despite antiemetic treatment. *J Clin Oncol.* 2006;24(27):4472-4478.
5. Davidson W, Teleni L, Muller J, et al. Malnutrition and chemotherapy-induced nausea and vomiting: implications for practice. *Oncol Nurs Forum.* 2012;39:E340 - 345.
6. Van Laar ES, Desai JM, Jatoi A. Professional educational needs for chemotherapy-induced nausea and vomiting (CINV): multinational survey results from 2388 health care providers. *Support Care Cancer.* 2015;23(1):151-157.
7. Cohen L, de Moor CA, Eisenberg P, Ming EE, Hu H. Chemotherapy-induced nausea and vomiting: incidence and impact on patient quality of life at community oncology settings. *Support Care Cancer.* 2007;15(5):497-503.
8. Marx WM, Teleni L, McCarthy AL, et al. Ginger (Zingiber officinale) and chemotherapy-induced nausea and vomiting: a systematic literature review. *Nutr. Rev.* 2013;71(4):245-254.
9. Marx W, Ried K, McCarthy AL, et al. Ginger-Mechanism of Action in Chemotherapy-induced Nausea and Vomiting: A Review. *Crit Rev Food Sci Nutr.* 2015:0.
10. Viljoen E, Visser J, Koen N, Musekiwa A. A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting. *Nutr J.* 2014;13:20.
11. Chaiyakunapruk N, Kitikannakorn N, Nathisuwan S, Leeprakobboon K, Leelasettagool C. The efficacy of ginger for the prevention of postoperative nausea and vomiting: a meta-analysis. *Am J Obstet Gynecol.* 2006;194(1):95-99.
12. Isenring E, Cross G, Kellett E, Koczwara B, Daniels L. Nutritional status and information needs of medical oncology patients receiving treatment at an Australian public hospital. *Nutrition and Cancer.* 2010;62(2):220-228.

13. Molassiotis A, Brearley SG, Stamataki Z. Use of antiemetics in the management of chemotherapy-related nausea and vomiting in current UK practice. *Support Care Cancer.* 2011;19(7):949-956.
14. Gardiner P, Sadikova E, Filippelli AC, White LF, Jack BW. Medical reconciliation of dietary supplements: don't ask, don't tell. *Patient Educ Couns.* 2015;98(4):512-517.
15. Mandal P, Das A, Majumdar S, Bhattacharyya T, Mitra T, Kundu R. The efficacy of ginger added to ondansetron for preventing postoperative nausea and vomiting in ambulatory surgery. *Pharmacognosy research.* 2014;6(1):52-57.
16. Lindley CM, Hirsch JD, O'Neill CV, Transau MC, Gilbert CS, Osterhaus JT. Quality of life consequences of chemotherapy-induced emesis. *Qual Life Res.* 1992;1(5):331-340.
17. Osoba D, Zee B, Warr D, Latreille J, Kaizer L, Pater J. Effect of postchemotherapy nausea and vomiting on health-related quality of life. The Quality of Life and Symptom Control Committees of the National Cancer Institute of Canada Clinical Trials Group. *Support Care Cancer.* 1997;5(4):307-313.
18. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ.* 2010;340.
19. Hassaine G, Deluz C, Grasso L, et al. X-ray structure of the mouse serotonin 5-HT3 receptor. *Nature.* 2014;512(7514):276-281.
20. Schwertner HA, Rios DC. High-performance liquid chromatographic analysis of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in ginger-containing dietary supplements, spices, teas, and beverages. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007;856(1-2):41-47.
21. van Breemen RB, Tao Y, Li W. Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale). *Fitoterapia.* 2011;82(1):38-43.
22. Chen C-Y, Cheng K-C, Chang AY, Lin Y-T, Hseu Y-C, Wang H-M. 10-Shogaol, an Antioxidant from Zingiber officinale for Skin Cell Proliferation and Migration Enhancer. *International Journal of Molecular Sciences.* 2012;13(2):1762-1777.
23. Academy of Nutrition and Dietetics, . Evidence Analysis Manual: Steps in the ADA Evidence Analysis Process. American Dietetic Association. 2012.
24. Morrow GR, Ballatori E, Groshen S, Olver I. Statistical considerations in the design, conduct and analyses of antiemetic clinical trials. An emerging consensus. *Support Care Cancer.* 1998;6(3):261-265.
25. Tonato M, Roila F, Del Favero A. Methodology of antiemetic trials: a review. *Ann Oncol.* 1991;2(2):107-114.
26. Olver IN, Simon RM, Aisner J. Antiemetic studies: a methodological discussion. *Cancer Treat Rep.* 1986;70(5):555-563.
27. Rothwell PM. External validity of randomised controlled trials: "to whom do the results of this trial apply?". *Lancet.* 2005;365(9453):82-93.
28. Manusirivithaya S, Sripramote M, Tangjitgamol S, et al. Antiemetic effect of ginger in gynecologic oncology patients receiving cisplatin. *Int J Gynecol Cancer.* 2004;14(6):1063-1069.

29. Fahimi F, Khodadad K, Amini S, Naghibi F, Salamzadeh J, Baniasadi S. Evaluating the Effect of Zingiber Officinalis on Nausea and Vomiting in Patients Receiving Cisplatin Based Regimens. *Iran J Pharm Res.* 2011;10(2):379-384.
30. Panahi Y, Saadat A, Sahebkar A, Hashemian F, Taghikhani M, Abolhasani E. Effect of ginger on acute and delayed chemotherapy-induced nausea and vomiting: a pilot, randomized, open-label clinical trial. *Integr Cancer Ther.* 2012;11(3):204-211.
31. Oh HS, Seo WS. Systematic review and meta-analysis of the correlates of cancer-related fatigue. *Worldviews Evid Based Nurs.* 2011;8(4):191-201.
32. Molassiotis A, Coventry PA, Stricker CT, et al. Validation and psychometric assessment of a short clinical scale to measure chemotherapy-induced nausea and vomiting: the MASCC antiemesis tool. *J Pain Symptom Manage.* 2007;34(2):148-159.
33. Lee YK, Georgiou C, Raab C. The knowledge, attitudes, and practices of dietitians licensed in Oregon regarding functional foods, nutrient supplements, and herbs as complementary medicine. *J Am Diet Assoc.* 2000;100(5):543-548.
34. Hetherwick C, Morris MN, Silliman K. Perceived knowledge, attitudes, and practices of California registered dietitians regarding dietary supplements. *J Am Diet Assoc.* 2006;106(3):438-442.
35. Sewitch MJ, Cepoiu M, Rigillo N, Sproule D. A Literature Review of Health Care Professional Attitudes Toward Complementary and Alternative Medicine. *Complementary Health Practice Review.* 2008;13(3):139-154.
36. NHMRC. NHMRC levels of evidence and grades for recommendations for developers of guidelines. Canberra: National Health and Medical Research Council; 2009.
37. Marx W, Kiss N, Isenring L. Is ginger beneficial for nausea and vomiting? An update of the literature. *Current Opinion in Supportive and Palliative Care.* 2015;9(2):189-195.
38. Yekta ZP, Ebrahimi SM, Hosseini M, et al. Ginger as a miracle against chemotherapy-induced vomiting. *Iran J Nurs Midwifery Res.* 2012;17(5):325-329.
39. Touger-Decker R, Thomson CA. Complementary and alternative medicine: Competencies for dietetics professionals. *Journal of the American Dietetic Association.* 2003;103(11):1465-1469.
40. Practice Paper of the American Dietetic Association: Dietary Supplements. *Journal of the American Dietetic Association.* 2005;105(3):460-470.
41. Vickery CE, Cotugna N. Complementary and Alternative Medicine Education in Dietetics Programs: Existent but Not Consistent. *Journal of the American Dietetic Association.* 2006;106(6):860-866.
42. Sontakke S, Thawani V, Naik MS. Ginger as an antiemetic in nausea and vomiting induced by chemotherapy: A randomized, cross-over, double blind study. *Indian J. Pharmacol.* 2003;35(1):32-36.

43. Zick S, Djuric Z, Ruffin M, et al. Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev.* 2008;17:1930 - 1936.
44. Yu Y, Zick S, Li X, Zou P, Wright B, Sun D. Examination of the pharmacokinetics of active ingredients of ginger in humans. *AAPS J.* 2011;13(3):417-426.
45. Pessi MA, Necchi A, Bossi P, et al. Nausea and vomiting during the first 3 intercycle periods in chemo-naive cancer patients receiving moderately/highly emetogenic therapy. *Tumori.* 2015;101(6):692-696.
46. Vogelzang NJ, Breitbart W, Cella D, et al. Patient, caregiver, and oncologist perceptions of cancer-related fatigue: results of a tripart assessment survey. The Fatigue Coalition. *Seminars in hematology.* 1997;34(3 Suppl 2):4-12.

Appendices

Appendices A. GRID Analysis and Structural Similarity Map

1) GRID RESULTS

a) Serotonin Site

Probe	Description	LEAU	MOVE	Most Neg pt Kcal/mol	NZ	X	Y	Z	Nearest residue
OH2	Water	2	0	-20	106	170.587	184.903	266.820	Ser150
C1=	Aromatic CH	0	0	-5.6799	84	163.153	182.237	259.487	Trp63
C3	Methyl C	0	0	-6.0628	84	163.153	182.237	259.487	Trp63
O1	Alkyl hydroxyl	1	0	-15.6125	23	165.487	172.903	239.153	Leu12
OH	Phenol hydroxyl	1	0	-12.9040	28	168.153	170.903	240.820	Asp162
O	Carbonyl O	1	0	-10.2049	23	165.820	172.903	239.153	Val27
DRY	Hydrophobic	0	0	-2.9457	115	171.820	192.903	269.820	Leu137
BOTH	Amphipathic	0	0	-1.4328	80	164.820	176.903	258.153	Trp156

b) Allosteric Site

Probe	Description	LEAU	MOVE	Most Neg pt Kcal/mol	NZ	X	Y	Z	Nearest residue
OH2	Water	2	0	-19.7242	49	170.393	184.930	266.750	GLU B 102
C1=	Aromatic CH	0	0	-5.7054	27	163.060	182.263	259.417	ASN B 101
C3	Methyl C	0	0	-6.1074	27	163.060	182.263	259.417	ASN B 101
O1	Alkyl hydroxyl	1	0	-15.7207	5	172.393	176.263	252.083	ALA B 208
OH	Phenol hydroxyl	1	0	-11.9311	15	176.060	200.930	264.750	ILE B 48
O	Carbonyl O	1	0	-10.0362	15	176.060	200.930	264.750	ILE B 48

DRY	Hydrophobic	0	0	-3.5633	20	172.060	192.930	269.750	LEU B 58
BOTH	Amphipathic	0	0	-1.1253	24	179.060	187.930	273.750	ARG B 65

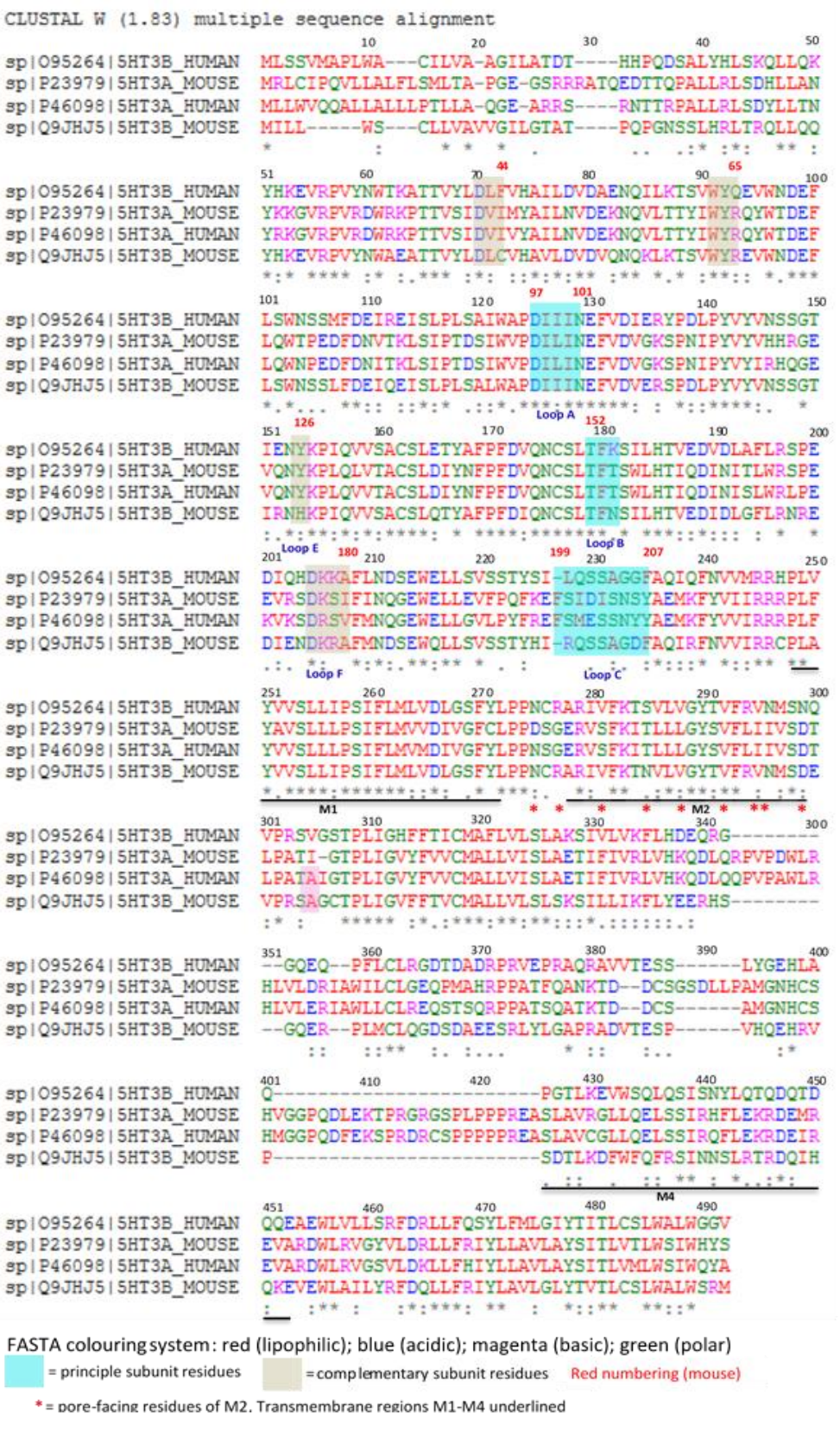
(2) Colour key to Structural Similarity Map for Serotonin Site

Cyan	4.246	4.84283
Blue	4.84283	5.43965
Purple	5.43965	6.03648
Violet	6.03648	6.63331
Green-Blue	6.63331	7.23013
Green	7.23013	7.82696
Yellow	7.82696	8.42379
Orange	8.42379	9.02062
Red-Orange	9.02062	9.61744
Red	9.61744	10.2143
Magenta	10.2143	10.8111

Colour key to Structural Similarity Map for Allosteric Site

Cyan	4.1193	4.58421
Blue	4.58421	5.04912
Purple	5.04912	5.51403
Violet	5.51403	5.97894
Green-Blue	5.97894	6.44384
Green	6.44384	6.90875
Yellow	6.90875	7.37366
Orange	7.37366	7.83857
Red-Orange	7.83857	8.30348
Red	8.30348	8.76839
Magenta	8.76839	9.2333

Appendices B. Fasta Sequencing of Murine and Human 5-HT_3 Receptor


CLUSTAL W (1.83) multiple sequence alignment
sp|O95264|5HT3B_HUMAN
sp|P23979|5HT3A_MOUSE
sp|P46098|5HT3A_HUMAN
sp|Q9JHJ5|5HT3B_MOUSE
Loop A
Loop B
Loop C
Loop E
Loop F
M1
M2
M4
FASTA colouring system: red (lipophilic); blue (acidic); magenta (basic); green (polar)
= principle subunit residues
= complementary subunit residues
Red numbering (mouse)
* = pore-facing residues of M2. Transmembrane regions M1-M4 underlined

Appendices C. CONSORT Diagram

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	Title page
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	282
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	284
	2b	Specific objectives or hypotheses	285
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	285
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	286
	4b	Settings and locations where the data were collected	285
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	287
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	288
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	292
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation: Sequence generation	8a	Method used to generate the random allocation sequence	287
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	287

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	287
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	287
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	287
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	292
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	292
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	300
	13b	For each group, losses and exclusions after randomisation, together with reasons	300
Recruitment	14a	Dates defining the periods of recruitment and follow-up	286
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	293
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	292
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	294
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	294
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	299
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	300
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	300
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	300

Other information			
Registration	23	Registration number and name of trial registry	285
Protocol	24	Where the full trial protocol can be accessed, if available	285
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	306

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Appendices D. Ethics Approval

BOND UNIVERSITY
BRINGING AMBITION TO LIFE

HUMAN RESEARCH ETHICS COMMITTEE
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ABN 88 010 694 121
CRICOS CODE 00017B

21 February 2014

Liz Isenring and Wolf Marx
Faculty of Health Science and Medicine
Bond University

Dear Liz and Wolf

Protocol No: **RO 1789**
Project Title: **Can ginger ameliorate chemotherapy-induced nausea and vomiting? A double-blind, randomised, placebo controlled trial**

I am pleased to confirm that your project was reviewed by the Chair of BUHREC under section 5.3 of the National Statement, which advises against duplication of review, and you have been granted approval to proceed which has now been ratified by the Committee.

As a reminder, BUHREC's role is to monitor research projects until completion. The Committee requires, as a condition of approval, that all investigations be carried out in accordance with the National Health and Medical Research Council's (NHMRC) National Statement on Ethical Conduct in Research Involving Humans and Supplementary Notes. Specifically, approval is dependent upon your compliance, as the researcher, with the requirements set out in the National Statement and listed in the Declaration which you have signed.

Please be aware that the approval is given subject to the protocol of the study being undertaken as described in your application with amendments, where appropriate. As you may be aware the Ethics Committee is required to annually report on the progress of research it has approved. We would greatly appreciate if you could advise us when you have completed data collection and when the study is completed.

You are reminded that the Principal Investigator must immediately report anything that might warrant review of ethical approval of the project.

Should you have any queries or experience any problems, please contact early in your research project: Telephone: (07) 559 53554, Facsimile: (07) 559 51120, Email: buhrec@bond.edu.au.

We wish you well with your research project.

Yours sincerely

Dr Mark Bahr
Chair

Appendices E. Patient Information and Withdrawal Form

Participant Information Sheet/Consent Form
Interventional Study - *Adult providing own consent*

Princess Alexandra Hospital

Title	*Can ginger ameliorate chemotherapy-induced nausea? A double blind, randomised placebo controlled feasibility study.*
Short Title	*2012 CINV Ginger RCT*
Protocol Number	*V3*
Project Sponsor	*Health Practitioners Research Scheme*
Coordinating Principal Investigator/ Principal Investigator	*Dr Elizabeth Isenring*
Associate Investigator(s)	Wolfgang Marx and Leigh Frazer
Location	*Princess Alexandra Hospital*

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project. This is because you have recently or are about to *commence chemotherapy*. The research project is testing a new treatment for *chemotherapy-induced nausea and vomiting*. The new treatment is an **extract of ginger** used as well as standard anti-nausea medication.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:
• Understand what you have read
• Consent to take part in the research project

• Consent to have the tests and treatments that are described
• Consent to the use of your personal and health information as described.
You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

Despite advances in anti-vomiting medication, nausea continues to be a problem for many patients receiving chemotherapy. Nausea may decrease quality of life and interfere with treatment. There is evidence from international studies that ginger capsules, when used with standard anti-vomiting medication, may help in the treatment of nausea that may occur during chemotherapy. However, this therapy is a relatively new treatment and therefore, not routinely used in chemotherapy centres. Additionally, there is a lack of information about how chemotherapy patients will tolerate ginger.

The aim of this study is to determine: 1) the effect of ginger on chemotherapy-induced nausea; 2) the tolerability of ginger to chemotherapy patients when used alongside standard anti-nausea medication; and 3) the feasibility of introducing it in our clinical setting.

- Ginger extract is an experimental treatment. This means that it is not an approved treatment for chemotherapy-induced nausea and vomiting in Australia.
- The results of this research will be used by the researcher Mr Wolfgang Marx to obtain a Doctor of philosophy (PhD) degree.
- This research has been initiated by the study doctor, Dr Elizabeth Isenring.
- This research has been funded by Health Practitioner Research Scheme.

3 What does participation in this research involve?

You will be participating in a randomised controlled research project. Sometimes we do not know which treatment is best for treating a condition. To find out, we need to compare different treatments. We put people into groups and give each group a different treatment. The results are compared to see if one is better. To try to make sure the groups are the same, each participant is put into a group by chance (random; like the toss of a coin).

- Prior to the study, an investigator will ask you a short number of questions relating to your age, history of nausea, and medical background to determine if you are eligible for participation in this trial. This will take approximately 5 minutes.
- Following this, you will be assigned to either receive a ginger extract or placebo. The trial will commence during your chemotherapy session, during which, you will be asked to take the study capsules (ginger or placebo) 4 times per day for 5 days: starting the day you have chemotherapy, and then continued on the four days post-chemotherapy.
- During this time, you will be given a booklet that contains a series of short, anonymous questionnaires that we ask you to complete. These questionnaires will primarily assess your quality of life and experience of nausea and vomiting symptoms. Your nutrition status will also be assessed by a dietitian during a short interview.
- This study will not interrupt your regular chemotherapy schedule, you will not be required to spend additional time at the hospital and no blood or tissue samples will be required.

- Your medical chart will be accessed to obtain details such as your year of birth, date of hospital admission, and diagnosis and will be recorded against a study number to ensure confidentiality.
- A placebo is a medication with no active ingredients or a procedure without any medical benefit. It looks like the real thing but is not.
- This research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions.

Please note: if you enrol in this study, you will still receive standard anti-emetic medication. You will not be excluded from any anti-emetic treatment, the supplements will instead be consumed on top of the medication prescribed by your medical team.

There are no additional costs associated with participating in this research project, nor will you be paid. All medication, tests and medical care required as part of the research project will be provided to you free of charge.

It is desirable that your local doctor be advised of your decision to participate in this research project. If you have a local doctor, we strongly recommend that you inform them of your participation in this research project.

4 What do I have to do?

- You will be required to take the study capsule (ginger or placebo) four times daily for 5 days during your first chemotherapy session. During this time, you will complete a series of short questionnaires with the help of the research assistant; this will occur the day of your chemotherapy and 4 days after. During the study period, do not consume large quantities of ginger-containing food items or supplements (other than the study supplement). Similarly, please do not employ any other anti-nausea therapies (such as herbal remedies, acupuncture, vitamin supplements) during this trial. If you think you have done any of these things, please inform the research team.
- If you begin anti-coagulant therapy (eg, warfarin) during this trial, alert your doctor of your participation in this trial.

5 Other relevant information about the research project

- We are aiming for approximately 120 people to be enrolled in this study.
- This study will only be run at the Princess Alexandra Hospital

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with *Princess Alexandra Hospital*.

7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this hospital. Other options are available; in this trial you may be receiving a ginger supplement *alongside* standard anti-nausea treatment; however, it is possible to receive only standard anti-nausea treatment, if you do not wish to participate in this study. Your study doctor will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, it is hoped that the knowledge gained from this study will help the cancer patient community by helping design effective anti-nausea care to decrease the burden of chemotherapy and to improve the quality of life of patients. Additionally, participants that are assigned to receive the ginger extract may experience a reduction in nausea and vomiting associated with chemotherapy; however, this is not guaranteed.

All efforts will be taken to conduct the trial in a manner that does not interrupt the daily routine of the patient. This includes conducting all assessments and follow-ups in proximity to patients' usual medical meetings to decrease transport time.

The Research Assistant will notify dietitians of the hospital if you have been assessed as experiencing nutritional problems so that you can receive appropriate nutrition care.

A summary of the results as group data will be provided to the Department of Nutrition & Dietetics of Princess Alexandra hospital and will be available to you on request.

9 What are the possible risks and disadvantages of taking part?

- Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects.
- Ginger has been used in many previous studies with minimal side effects reported; however, formal testing of the safety profile of ginger has not yet been conducted. Potential side effects that have been previously reported have been mild in nature and include the development of a rash, gastrointestinal discomfort, and heartburn.

- Ginger may interact with medication used to treat blood clots such as warfarin; however, all participants will be screened at the commencement of the study to ensure they are not currently prescribed any of these medications. If you undergo anti-coagulant therapy or are prescribed warfarin during this trial, you will be withdrawn from the study for your own safety.
- There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.
- Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you.
- At no time will your personal medical details be used in a way so as to have you identified by a third party. Your confidentiality will at all times be safeguarded.
- The effects of *ginger extract* on the unborn child and on the newborn baby are not known. Because of this, it is important that research project participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project. You must not participate in the research if you are pregnant or trying to become pregnant, or breast-feeding.
- If you do become pregnant whilst participating in the research project, you should advise your study doctor immediately. Your study doctor will withdraw you from the research project and advise on further medical attention should this be necessary. You must not continue in the research if you become pregnant.

10 What will happen to my test samples?

- No blood or tissue samples will be required for this study.
- All questionnaire data will be stored securely to maintain participant confidentiality.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments, particularly if you are taking them for nausea,

vomiting or any other gastrointestinal symptoms. You should also tell your study doctor about any changes to these during your participation in the research project. Your study doctor should also explain to you which treatments or medications need to be stopped for the time you are involved in the research project.

13 What if I withdraw from this research project?

You are free to withdraw at any time. If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the sponsor up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as:
- Unacceptable side effects
- The treatment being shown not to be effective
- The treatment being shown to work and not need further testing

15 What happens when the research project ends?
The study will provide the study capsules for the duration of the study; however, they will not be provided after the study ends. Participants will, however, continue standard anti-nausea medication.

Part 2: How is the research project being conducted?

16 What will happen to information about me?
- By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. All data sheets will be stored in a patient file in a locked filing cabinet in the Department of Nutrition & Dietetics, Princess Alexandra Hospital. The patient files will be de-identified and given an individual study code to ensure that all information remains confidential. Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.
- Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

- It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. No personal or identifiable data will be presented or published, only general results by group will be presented.
- Information about your participation in this research project may be recorded in your health records.
- In accordance with relevant Australian *and/or Queensland* privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.
- Any information obtained for the purpose of this research project that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.
- If you would like a copy of the study results, you can request this by contacting the research team.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

18 Who is organising and funding the research?

- This research project is being conducted by Dr Elizabeth Isenring. Funding for this study was obtained through the Health Practitioners Research Scheme grant.
- No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).
- Study medications for the project have been purchased from the nutritional supplement company, Bluebonnet Nutrition. However, this company will have no involvement in the study design, collection, analysis or interpretation of the data, writing the report, or the decision to submit any manuscript for publication.

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of Princess Alexandra Hospital

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study investigator on *3365 6982* or any of the following people:

Clinical contact person

Name	*Dr Elizabeth Isenring*
Position	*Principle Investigator*
Telephone	
Email	

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Position	Patient Liaison Officer
Telephone	
Email	*PAH_PLO@health.qld.gov.au*

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	*Metro South Hospital and Health Service HREC*
Telephone	
Email	*PAH_Ethics_Research@health.qld.gov.au*

Local HREC Office contact (Single Site -Research Governance Officer)

Name	*Metro South Hospital and Health Service HREC*
Position	*HREC Coordinator*
Telephone	
Email	*PAH_Ethics_Research@health.qld.gov.au*

Consent Form - *Adult providing own consent*

Title	*Can ginger ameliorate chemotherapy-induced nausea? A double blind, randomised placebo controlled feasibility study*
Short Title	*2012 CINV Ginger RCT*
Protocol Number	*V3*
Project Sponsor	*Health Practitioners Research Scheme*
Coordinating Principal Investigator/ Principal Investigator	*Dr Elizabeth Isenring*
Associate Investigator(s)	Wolfgang Marx and Leigh Frazer
Location	*Princess Alexandra Hospital*

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to *Princess Alexandra Hospital* concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please _____

Signature _____ Date _____

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	*Can ginger ameliorate chemotherapy-induced nausea? A double blind, randomised placebo controlled feasibility study*
Short Title	*2012 CINV Ginger RCT*
Protocol Number	*V3*
Project Sponsor	*Health Practitioners Research Scheme*
Coordinating Principal Investigator/ Principal Investigator	*Dr Elizabeth Isenring*
Associate Investigator(s)	Wolfgang Marx and Leigh Frazer
Location	*Princess Alexandra Hospital*

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with *Princess Alexandra Hospital.*

Name of Participant (please _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

Appendices F. Survey Questions Plan

Demographic

Age

- <30
- 31-40
- 41-50
- 51-60
- >61

What is your profession?

- Dietitian
- Doctor
- Surgeon
- Allied Health Professional
- Nurse
- Psychiatrist
- Psychologist
- Other

How many years have you worked as in your profession?0-2

- 2-5
- 5-10
- 10-15
- 15-25
- >25

What is your highest level of education?

- Diploma
- Bachelor
- Masters
- PhD

Job area (greatest time spent in your current position)

- Acute care
- Community
- Private practise
- Industry
- Other _____

Approximately how much of your workload is spent consulting with cancer patients?

- 81-100%
- 50-80%
- <50%

Do you sell supplements as part of your clinical practise?

Do you consider this a conflict of interest?

Do you feel you have any other potential conflicts of interest that may bias your response to this survey? For example, profit gain from advertising specific supplements.

Attitudes regarding dietary supplements

Please describe how much you agree or disagree with the following statements regarding dietary supplements.

Strongly agree, agree, neutral, disagree, strongly disagree.

- I am knowledgeable about dietary supplements.
- I am interested in dietary supplements.
- People in your profession are knowledgeable about dietary supplements
- I was well trained in dietary supplements.
- This area is important to improving health outcomes.
- Dietary supplements are effective.
- <response to Q3> should be knowledgeable about dietary supplements.
- <response to Q3> should be considered an authority on dietary supplements.
- There is a high demand for dietary supplements.
- I am often asked about dietary supplements by patients or clients.

- I feel confident in answering questions regarding dietary supplements.
- I am interested in further training on dietary supplements.
- Dietary supplements are safe.
- <response to Q3> should play a greater role in the prescription of dietary supplements.
- <response to Q3> should play a greater role in the education regarding the use of dietary supplements.
- <response to Q3> should play a greater role in research regarding the use of dietary supplements.
- I think universities should offer more training in these areas as part of their curriculum.
- I am able to access trustworthy information regarding dietary supplements.
- I regularly recommend dietary supplements to clients/patients.

How often do you personally take one or more dietary supplement?

- Daily/most days/occasionally/never

Support for position

Please answer how strongly the following groups of people would agree or disagree with your position on these therapies.

- Doctors
- Dietitians
- Your professions governing body (i.e DAA for Australian dietitians)General public
- Pharmacists
- Nurses
- Naturopaths

For the general public, who do you believe **<u>are</u>** the primary sources of information regarding dietary supplements? (Tick as many as you feel suitable)

- Doctors
- Pharmacists

- Naturopaths
- Dietitians
- Nurses
- Friends and family
- Television/radio
- Internet
- Other

For the general public, who **should** be the primary sources of information regarding dietary supplements? (Tick as many as you feel suitable)

- Doctors
- Pharmacists
- Naturopaths
- Dietitians
- Nurses
- Friends and family
- Television/radio
- Internet
- Other

Where do you get your information regarding dietary supplements? (Tick as many as you feel suitable)

- Conferences
- Workshops
- Colleagues
- Friends and family
- Evidence databases (e.g PEN library)
- DAA guidelines or other official guidelines
- Television/radio
- Internet
- Books
- Academic journals

What is the **minimum** level of evidence that you require before you would feel confident utilising or recommending specific dietary supplements in your workplace? Please select only one response.

- Cell culture and lab research
- Animal studies
- Case studies
- Observational and epidemiological studies
- Non-blinded, open label human trials
- Randomised control trials
- Meta-analysis
- Published guidelines
- Other

If you selected other, could you please elaborate on this?

<Answer box>

In relation to your answer to the previous question, approximately how many of these studies/guidelines would need to be published before you utilise specific dietary supplements?

- One
- Two to four
- Five or more

What area do you think dietary supplements are most effective for? Please tick as many as you feel necessary.

- Sports performance
- Acute-care (e.g. cancer cachexia, post-operative recovery)
- Cancer prevention

- Cancer treatment
- Symptom management (e.g. nausea, fatigue)
- Prevention of other chronic diseases (e.g cholesterol management)
- Management and treatment of other chronic diseases (e.g. CVD, T2DM)
- Weight loss
- Digestive disorders (e.g. IBS, Chrons disease)
- Mental and cognitive issues (e.g. stress)
- Sleep disorders
- Other
- Dietary supplements are not effective for any area

If you selected other, could you please elaborate on this?

<Answer box>

What do you feel are the major barriers to you recommending the use of dietary supplements to your patients/clients? Please tick as many as you feel necessary.

- A lack of training in this area
- A lack of confidence in this area
- Concerns regarding potential interactions with other treatments
- Concerns regarding potential negative effects of dietary supplements
- Concerns about the regulation of dietary supplements
- Perceived lack of efficacy of dietary supplements
- It may conflict with the advice of the patients/clients medical team
- Lack of authority to recommend dietary supplements to patients/clients
- A lack of interest in this area
- Concerns regarding financial burden on patient
- Perceived Lack of quality dietary supplements on the market
- No barriers, I recommend the use of dietary supplements.
- Other

If you selected other, could you please elaborate on this?

<Answer box>

Do you have any suggestions to address these barriers?

What do you feel are the major enablers to you recommending the use of dietary supplements to your patients/clients? Please tick as many as you feel necessary.

- I have sufficient training in this area
- There are sufficient regulations regarding dietary supplements
- There is sufficient research to show the efficacy of dietary supplements
- There is sufficient research to show the safety of dietary supplements
- The physicians and medical team of patient/client are supportive of the use of dietary supplements
- I have sufficient autonomy to recommend dietary supplements to patients/clients
- Dietary supplements are cost-effective.
- There are high-quality supplements available on the market.
- No enablers, I do not currently recommend the use of dietary supplements.
- Other

Which area would you like to learn more about? Please tick as many as you feel suitable.

- Specific dietary supplements
  - If so, could you please specify? Please list as many supplements as you feel necessary.
- The usage of dietary supplements for specific diseases (e.g. cancer) or goals (e.g. sports performance)
- Drug-supplement interactions
- Regulatory issues regarding dietary supplements
- Reliable sources of information regarding dietary supplements
- Adverse effects of dietary supplements
- Other
  - Could you please specify?

Do you wish to say anything else that was not covered in the previous questions?

Conclusion

You have completed our survey, we sincerely thank you for your input!

If you have any questions regarding this project, please the principal investigator, Liz Isenring (lisenrin@bond.edu.au).

Should you have any complaints concerning the manner in which this research is being conducted please make contact with:

Bond University Human Research Ethics Committee, c/o Bond University Office of Research Services.
Bond University, Gold Coast, 4229

Tel: +61 7 5595 4194 Fax: +61 7 5595 1120 Email: buhrec@bond.edu.au

Appendices G. Clinical Trial Evidence Appraisal

Quality Assessment Criteria of Included Clinical Trial

	Rating	Explanation
Quality Rating (+,0,-)	**Positive +**	
Year	2015	
Relevance Questions		
1 Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (NA for some Epi studies)	Yes	CINV is a serious clinical issue that affects a large proportion of the cancer population. Treatments to improve this control will significantly improve patient outcomes. Ginger supplementation is a widely-available, low-cost, and easy to administer potential intervention.
2 Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes	Interest in CAM therapies is high amongst cancer patients. Nausea and vomiting are highly distressing to patients undergoing treatment.
3 Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	Yes	Vomiting and in particular, nausea, are highly prevalent in this population, affecting up 60% of patients undergoing chemotherapy.
4 Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes	Ginger is a well-tolerated, widely-available intervention. Many previous studies have been conducted successfully.
Validity Questions		
1 Was the research question clearly stated?	**Yes**	
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	Yes	1.2g standardized ginger extract (4x300mg).
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	Yes	Primary outcome: Chemotherapy-induced nausea-related quality of life Secondary outcomes: see protocol chapter (chapter 8)

1.3 Were the target population and setting specified?	Yes	Chemotherapy-naïve patients commencing moderately or highly emetogenic chemotherapy regimens. Recruited from Princess Alexandra Hospital, Queensland, Australia
2. Was the selection of study subjects/patients free from bias?	**Yes**	
2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes	Patients were recruited if they were chemotherapy-naïve, were due to receive a moderately or highly emetogenic chemotherapy regimen, were at least 18 years old, had a baseline Karnofsky score >60, had no known concurrent neoplasms or illness that induces nausea independent of chemotherapy, and did not self-prescribe therapies or complementary products used for nausea. Patients were excluded if they met any of the following criteria: scheduled to receive radiotherapy during the study period, pregnant or lactating, concurrent use of other ginger-containing supplements and ingestion of large quantities of ginger, history of adverse reactions to ginger, and thrombocytopenia.
2.2 Were criteria applied equally to all study groups?	Yes	As stated in manuscript
2.3 Were health, demographics, and other characteristics of subjects described?	Yes	As shown in Figure 1 of manuscript
2.4 Were the subjects/patients a representative sample of the relevant population?	Yes	Gender, age, chemotherapy regimen were represented in equal proportions and is representative of the general cancer population
3 Were study groups comparable?	**Yes**	Y

3.1 Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes	Patients were randomised using a computer generated randomisation sequence
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes	As shown in Figure 1 of manuscript
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes	A parallel trial design was used for this study
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were pre-existing differences accounted for by using appropriate adjustments in statistical analysis?	N/A	
3.5 If case control study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A	
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A	
4 Was method of handling withdrawals described?	**Yes**	
4.1 Were follow up methods described and the same for all groups?	Yes	All patients were followed up at the end of each chemotherapy cycle as well as at the commencement of cycle 2 and 3. This was consistent across all patients

4.2 Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes	This was included in the study flow diagram, table X and discussed in the discussion section of the manuscript
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	Yes	All dropouts were documented with given reason
4.4 Were reasons for withdrawals similar across groups?	N	Reasons for dropout were varied and no consistent pattern between groups was apparent Dropouts were slightly higher in the placebo group
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A	
5 Was blinding used to prevent introduction of bias?	**Yes**	Y
5.1 In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes	All patients were blinded as well as all research staff involved in the recruitment process
5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	N	Outcome was self-reported. Due to the nature of the outcomes, blinding of the outcome is not possible.
5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A	
5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A	

5.5 In diagnostic study, were test results blinded to patient history and other test results?	N/A	
6 Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?	**Yes**	
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	Yes	All details are included in methods section as well as the trial protocol paper.
6.2 In observational study, were interventions, study settings, and clinicians/provider described?	N/A	
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes	Patients were followed during the time period (first 5 days of chemotherapy) where CINV is likely to occur. Dosage of intervention is in line with preliminary clinical and pre-clinical evidence.
6.4 Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes	Patients were asked to record their compliance. Patients were also interviewed at the end of each cycle to assess compliance and blinding
6.5 Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes	Anti-emetic use and chemotherapy regimen was recorded
6.6 Were extra or unplanned treatments described?	N/A	
6.7 Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes	
6.8 In diagnostic study, were details of test administration and replication sufficient?	N/A	
7. Were outcomes clearly defined and the measurements valid and reliable?	**Yes**	
7.1 Were primary and secondary endpoints described and relevant to the question?	Yes	

7.2 Were nutrition measures appropriate to question and outcomes of concern?	Yes	PG-SGA is a validated questionnaire to assess nutrition status. This was administered by an accredited practising dietitian.
7.3 Was the period of follow-up long enough for important outcome(s) to occur?	Yes	
7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes	All questionnaires were validated and have been widely used in the cancer setting
7.5 Was the measurement of effect at an appropriate level of precision?	Yes	
7.6 Were other factors accounted for (measured) that could affect outcomes?	Yes	Influence of multiple prognostic factors (e.g. age, emetogenicity, anticipatory CINV) were assessed
7.7 Were the measurements conducted consistently across groups?	Yes	
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?	**Yes**	
8.1 Were statistical analyses adequately described the results reported appropriately?	Yes	
8.2 Were correct statistical tests used and assumptions of test not violated?	Yes	
8.3 Were statistics reported with levels of significance and/or confidence intervals?	Yes	
8.4 Was “intent to treat” analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes	

8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes	
8.6 Was clinical significance as well as statistical significance reported?	Yes	
8.7 If negative findings, was a power calculation reported to address type 2 error?	Yes	
9. Are conclusions supported by results with biases and limitations taken into consideration?	**Yes**	
9.1 Is there a discussion of findings?	Yes	
9.2 Are biases and study limitations identified and discussed?	Yes	
10. Is bias due to study's funding or sponsorship unlikely?	**Yes**	
10.1 Were sources of funding and investigators' affiliations described?	Yes	
10.2 Was there no apparent conflict of interest?	Yes	