THE EFFECT OF *CARUM CARVI* WATER EXTRACT INTAKE AS AN ALTERNATIVE THERAPY FOR WEIGHT LOSS IN OVERWEIGHT AND OBESE WOMEN

MAHNAZ KAZEMIPOOR

FACULTY OF SCIENCE

UNIVERSITY OF MALAYA

KUALA LUMPUR

2014

THE EFFECT OF *CARUM CARVI* WATER EXTRACT INTAKE AS AN ALTERNATIVE THERAPY FOR WEIGHT LOSS IN OVERWEIGHT AND OBESE WOMEN

MAHNAZ KAZEMIPOOR

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF SCIENCE & TECHNOLOGY STUDIES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2014

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **Mahnaz Kazemipoor** (I.C/Passport No: **X95386397**)

Registration/Matric No: **SHB110005**

Name of Degree: **Doctor of Philosophy (PhD)**

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

The Effect of *Carum carvi* Water Extract Intake as an Alternative Therapy for Weight Loss in Overweight and Obese Women

Field of Study: **Nutrition & Traditional medicine**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

ABSTRACT

Obesity and overweight are considered as challenging health problems worldwide. Despite several modern methods for treatment of obesity such as medical nutrition therapy, low-energy diets, and physical activity. The prevalence of this disease is still high, suggesting the need for alternative therapies. One of the trending approaches is the consumption of traditional medicinal plants. Caraway (*Carum carvi L.*), has been traditionally used as a spice in Iran. It is also claimed as a potent medicinal plant that is used to treat a variety of ailments including obesity. The objectives of this study are to analyse the phytochemicals present in caraway water extract (CWE), to measure body composition, anthropometric indices, dietary food intake and appetite, and clinical and para-clinical parameters before and after twelve-weeks of intervention. Hence, a randomized triple blind placebo controlled clinical trial was carried out on healthy, overweight and obese adult women in Yazd, Iran. The phytochemical content of CWE was analysed using the gas chromatography-mass spectrometry (GC-MS) technique. Out of 110 volunteers registered, 70 eligible candidates were randomized into two groups of caraway treatment and placebo (N=35 in each group) and were assigned to participate in a twelve-week intervention. Data were collected through questionnaire, face-to-face interview, physical examination and biochemical tests. Body composition and anthropometric indices were measured using bioelectrical impedance analyser (BIA) and measuring tape, while the appetite index was assessed using visual analogue scale method (VAS). Participants received either 30mL/day of CWE or placebo without changing their diet or physical activity and were examined at baseline and after twelve weeks of intervention and the data were analysed using SPSS. Results showed that the predominant ingredients detected from the GC-MS analysis were of different volatile and phenolic

compounds, including limonene, terpinene, carveol, carvone, carvacrol, and thymol. After twelve weeks of study results showed significant reduction of weight (-1.9 kg), body mass index (-0.8 kg/m2), body fat percentage (-0.7%), appetite level (-1) and carbohydrate intake (-30g) in the CWE group. All anthropometric indices including waist circumference, waist to hip ratio, thigh circumference and mid-upper arm circumference also reduced significantly (-6.2, -0.1, -5.4, and -2.2 cm respectively). Besides, significant increase was observed in the muscle percentage of the CWE group (+0.2%). No changes were detected in blood and urine tests, blood pressure and heart rate of respondents. Moreover, after twelve weeks of study, the red blood cell (RBC) level showed a clinically significant rise (+0.3 106/µL), whereas the platelet distribution width (PDW) showed a significant drop in the CWE group (-1.8 fL). The results suggest that adding CWE into the daily diet with no restriction in food intake, when combined with exercise, is of value for obese and overweight women wishing to reduce their body weight, BMI, body fat percentage, body size and appetite and carbohydrate intake. In addition, CWE intake has also improved body muscle and RBC level of the subjects with no clinical side effects. In conclusion, the results of this study suggest a safe weight loss adjuvant and a potential phytotherapeutic approach for CWE in the management of obesity.

ABSTRAK

Obesiti dan berat badan berlebihan merupakan masalah kesihatan yang kian mencabar di seluruh dunia. Walaupun terdapat pelbagai kaedah terapi moden di pasaran tetapi ia masih pada tahap yang sukar untuk dilakukan. Sebagai cadangan, terapi alternatif iaitu dengan pengambilan atau penggunaan tumbuh-tumbuhan tradisional boleh dilakukan. Jintan (*Carum Carvi L.*) telah digunakan secara tradisional sebagai herba di Iran. Ia dinyatakan mempunyai potensi sebagai tumbuhan yang mempunyai nilai perubatan untuk mengatasi pelbagai masalah kesihatan termasuk menurunkan berat badan yang berkait rapat dengan obesiti. Objektif utama kajian ini adalah untuk menganalisa kandungan fitokimia yang terdapat dalam ekstrak air jintan (CWE), mengkaji kesan pengambilan CWE terhadap komposisi badan dan indeks antropometri, serta mengkaji selera makan responden. Analisa terhadapkandungan fitokimia CWE dilakukan dengan menggunakan teknik gas kromatografi–spektrometri jisim(GC-MS). Penentuan beberapa parameter secara klinikal dan para-klinikal sebelum dan selepas masa intervensi turut dilakukan kepada wanita Yazd, Iran terpilih, iaitu yang sihat dan mempunyai berat badan berlebihan serta obes. Ia dilakukan melalui ujian secara rawak `triple blind placebo'. Seramai 110 orang responden telah mendaftar tetapi hanya 70 responden sahaja dipilih untuk mengambil bahagian selama tiga bulan.. Mereka dibahagikan secara rawak kepada dua kumpulan iaitu yang mengambil CWE (n=35) dan kumpulan kawalan atau plasebo (n=35). Data diperolehi melalui soal selidik, temuduga bersemuka, pemeriksaan fizikal dan ujian biokimia. Komposisi badan dan indeks antropometri diukur dengan menggunakan Bioelectrical Impedance Analyzer (BIA) dan pita mengukur, manakala tahap selera makan pula dinilai dengan kaedah visual analogue scale (VAS). Responden akan diberi sama ada 30ml/hari CWE atau 30ml/hari plasebo. Responden kedua-dua

kumpulan ini tidak akan mengubah corak pemakanan atau aktiviti fizikal sepanjang kajian ini dijalankan. Responden akan dinilai sebelum dan selepas 3 bulan tempoh yang ditetapkan. Hasil kajian menunjukkan bahan utama yang dikesan daripada analisis GC - MS adalah sebatian yang berbeza kadar pemeruapan dan terdapat kumpulan fenolik seperti limonene, terpinene, carveol, carvone, carvacrol, dan thymol. Hasil kajian terhadap responden selepas 12 minggu, menunjukkan berat badan (-1.9 kg), indeks jisim (-0.8 kg/m2), peratusan lemak (-0.7%), selera makan (-1g) dan pengambilan karbohidrat (-30g) menurun bagi kumpulan CWE. Malah hasil juga menujukkan semua indeks antropometri iaitu lilitan pinggang, nisbah pinggang ke pinggul, tahap purata lilitan paha dan lilitan pertengahan atas lengan ke pinggul turut berkurangan bagi kumpulan CWE (-6.2, -0.1, -5.4, dan -2.2cm). Peningkatan peratusan otot yang signifikan turut berlaku bagi kumpulan CWE (+0.2%). Seterusnya, tiada perubahan dikesan dalam darah, air kencing, tekanan darah dan kadar denyutan jantung responden bagi kumpulan CWE. Kajian juga mendapati tahap bilangan sel darah merah (RBC) meningkat (+0.3 106/µL) manakala julat taburan platelet (PDW) menurun bagi kumpulan CWE (-1.8 fL) selepas 12 minggu. Didapati dengan menambah CWE kedalam pemakanan harian dan digabungkan dengan senaman, kesan yang lebih signifikan diperolehibagi wanita obes dan mempunyai berat badan berlebihan untuk menurunkan berat badan, BMI, peratusan lemak badan dan , menurunkan selera makan. Pengambilan CWE juga dibuktikan secara klinikal dapat meningkatkan otot badan dan sel darah merah tanpa memberi kesan sampingan.Kesimpulannya, kajian menunjukkan terdapat pendekatan fitoterapi yang berpotensi bagi penggunaan CWE dalam mengatasi dan mengawal obesiti dan ia merupakan produk alternatif yang selamat digunakan.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor Dr. Che Wan Jasimah Bt wan Mohammed Radzi, and my co-supervisor, Associate Professor Dr. Majid Hajifaraji, for their useful comments, remarks and engagement through the learning process of this PhD thesis. In addition, a thanks to all academic staffs, and faculty members, for their support and guidance.

I would like to express the deepest appreciation to Professor Geoffrey A. Cordell, who has shown the attitude and the substance of a genius: whose valuable consultancy, encouragement, and contribution in stimulating suggestions, helped me to coordinate my thesis especially in writing articles. He continually and persuasively conveyed a spirit of adventure in regard to research and scholarship, and an excitement in regard to teaching. Without his support and constant help this thesis would have not been possible.

Furthermore, I would like to thank my parents, for their endless love, kindness and support they have shown during the past three years it has taken me to finalize this thesis. Last but not least, I would like to thank my friends for their assistance and support.

I would like to thank my loved ones, who have supported me throughout entire process, both by keeping me harmonious and helping me putting pieces together. I will be grateful forever for your love. I would like to express my deepest appreciation to all those who provided me the possibility to complete my PhD journey successfully.

TABLE OF CONTENTS

List of Figures

List of Tables

List of Symbols and Abbreviations

ALP	:	Alkaline phosphatase
ALT, SGPT	:	Alanine transaminase, glutamate pyruvate transaminase
AMR	:	Active metabolic rate
AST, SGOT	:	Aspartate transaminase, glutamate oxaloacetate transaminase
BF	:	Body fat
BIA	:	Bioelectrical impedance analyser
BM	:	Body muscle
BMI	:	Body mass index
BMR	:	Basic metabolic rate
BP	:	Blood Pressure
BW	:	Body water
CAM	:	Complementary and Alternative Medicine
CBC	:	Complete blood cell count
CHO	:	Carbohydrates
Chol/HDL	:	Cholesterol to HDL ratio
CWE	:	Caraway water extract
DBP	:	Diastolic blood pressure
FBS	:	Fasting blood sugar
FFQ	:	Food frequently questionnaire
FID	:	Flame ionization detector
g/kg	:	Gram/kilogram
GC-MS	:	Gas chromatography-mass spectrometry
GI	:	Gastrointestinal
GM	:	Gut microflora
HC	:	Hip circumference
HCT	:	Hematocrit
HDL-C	:	High density lipoprotein cholesterol
HGB	:	Hemoglobin
HPLC	:	High performance liquid chromatography
HR	:	Heart rate
IPAQ	:	International Physical Activity Questionnaire
IU/L	:	International unit per litre
LDL-C	:	Low density lipoprotein cholesterol
MCH	:	Mean corpuscular hemoglobin
MCHC	:	Mean corpuscular hemoglobin concentration
MCV	:	Mean corpuscular volume
MET	:	metabolic equivalent
mg/dL	:	Milligram/desi litre
MPV	:	mean platelet volume
MUAC	:	Mid-upper arm circumference
PAL	:	Physical activity level
PDW	:	Platelet distribution width
PLT	:	Platelets count
RBC	:	Red blood cell
RCT	:	Randomized clinical trial
RDW-CV	:	Red cell distribution width
REE	:	Resting energy expenditure
SBP	:	Systolic blood pressure
SD	:	Standard deviation
T&CM	:	Traditional and Complementary Medicine
TC	:	Thigh circumference
T-C	:	Total cholesterol
TDEE	:	Total daily energy expenditure
TG	:	Triglyceride
TM	:	Traditional Medicine
UA	:	Uric acid
UFA	:	Unsaturated fatty acids
USG	:	Urine-specific gravity
VAS	:	Visual analogue scale
VLDL	:	Very low density lipoprotein
WBC	:	White blood cell
WC	:	Waist circumference, ,
WHR	:	Waist to hip ratio

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Health issues have become an indispensable aspect of human life, and the importance of wellness and fitness in modern and emerging societies around the world is established. Global health has become a fundamental element of foreign policy and many governments now emphasize community health, and encourage institutions, researchers, and the media to develop, support and publicize research projects related to health promotion and wellness (Farr & Virchow, 2009). This greater need for health awareness among societies, brings focus to those factors which influence, positively or negatively, both individual and societal health.

Controlled clinical trials, which can assess the use of plant materials in the treatment and prevention of various diseases and human conditions are also needed, particularly when the existing therapeutic modalities are either not accessible or present a clinical risk. The World Health Organization (WHO) has recognized this for many years (World Health Organization, 2002) and has further encouraged countries to place the issues of both safety and efficacy as a priority in countries where traditional medicines and a variety of plant products are present in the health care market place (World Health Organization, 2014b).

Another factor which has now emerged as being crucial to medicinal plant research and development is sustainability, and the term "sustainable medicine" has been developed (Cordell, 2009, 2011a, 2011b; Cordell & Colvard, 2012) to describe the importance of considering the long-term use of traditional medicines (and synthetic drugs) from a perspective of reliable and non-destructive sourcing for the future. This is of particular importance where population use of traditional medicines is expanding, where the

globalization of products is increasing demand, or where climate change may impact areas for growing traditional medicines. In the research component of this scenario, preference is given to studies on those plant materials which are already established as sustainable commercial entities, or which are easily grown agronomically in order to derive an accessible (affordable and sustainable) product (Cordell, 2012). Another aspect with respect to the sustainability of traditional medicine applies to the knowledge of the use of medicinal plants, and how that information is recorded and maintained from generation to generation of practitioner.

One of the major global health problems that has emerged as a result of improved economic status, the globalization of certain eating practices, and personal health awareness, is overweight and obesity. Since 1997, the WHO has warned that obesity is rapidly becoming a global epidemic, although it was not a noticeable health care concern during most of the 20th century (Auld & Powell, 2009; Caballero, 2007; World Health Organization, 2000b) . The use of the word "Globesity" in reports indicates the severity of the issue worldwide (Delpeuch *et al.*, 2013).

The consequences of obesity to society in terms of morbidity and mortality are enormous. Based on recent research, obesity is implicated as one of the leading causes of death worldwide, and is a well-established, threatening element for human health (Barness *et al.*, 2007; Mokdad *et al.*, 2004). Furthermore, excess body fat can lead to the development of numerous, life-threatening, chronic diseases (Calle *et al.*, 2003; Guh *et al.*, 2009; Shehzad *et al.*, 2011).

In order to combat obesity and its health consequences, governments have adopted different policies which are essentially based on modifying lifestyle habits and increasing the health awareness of individuals. These programs mainly aim to promote healthy eating patterns and increase physical activity among people, especially school children. However, despite great efforts to fight this disease, "Globesity" remains a very challenging issue, and the management of obesity has become one of the crucial components of global and national health policies (Nestle & Jacobson, 2000).

Despite a variety of different treatment modern approaches for obesity, including surgery, weight loss pills, and dietary supplements, they do not satisfactorily impact weight loss, or are not tolerated by the body (Chaput *et al.*, 2007; Pittler *et al.*, 2005). In addition, the high costs and the side-effects of these methods, drive patients and researchers to seek alternative approaches (Clegg *et al.*, 2003; Pittler & Ernst, 2005) . Many scientists and patients believe that treatment with medicinal plants may provide a safer, more reliable, and also cheaper, approach to addressing issues of overweight and obesity, than the prevalent contemporary methods (Chang, 2000).

Based on literature review, different medicinal plants such as green tea, cinnamon, and turmeric have shown antiobesity activity which is evidenced scientifically (Hasani-Ranjbar *et al.*, 2013; Vermaak *et al.*, 2011; Yun, 2010). However, in spite of several studies on the application of traditional medicinal plants for managing body weight, many challenging issues, including the safety and efficacy of anti-obesity plants remain, and there are continuing deficiencies in the deployment of natural approaches for treating obesity (Jacobs & Gundling, 2009). Consequently, seeking sustainable, natural product-based solutions, and examining a variety of natural sources for methods to safely and

reliably treat obesity are important, albeit neglected, research targets. Success in developing such strategies will subsequently help to reduce the global health implications of obesity. This study describes an approach to establishing the safety and efficacy of a plant-based material, caraway water extract (CWE), for treating obesity and overweight, which might be considered as a natural alternative to the currently available dietary supplements and commercial products.

Caraway, particularly the fruit, is an ancient spice and flavoring material used in many parts of Europe, the Middle East, and Asia (Mariaca *et al.*, 1997). It is derived from the umbelliferous plant, *Carum carvi L*. (Apiaceae) (Hammer *et al.*, 1988), and is used world-wide as a natural flavoring in various food products, including rye bread, curries, to flavour rice, in sauerkraut, in cheeses, and as a liquor. Traditionally, caraway tends to be more widely used for weight loss purposes, especially in the countries of the Middle-East region. One of the reasons is historical. In Islamic traditional references, such as Khorasani's Makhzan al-adviyah (The Storehouse of Medicaments), and Avicenna's Canon of Medicine (980-1037 AD), the consumption of caraway aqueous extract is recommended specifically for weight loss (Aqili Khorasani, 2001; Nasser *et al.*, 2009).

Moreover, a number of the components present in caraway, including the polyphenols and specific essential oil components have been attributed to possess anti-inflammatory, anti-hyperlipidemic, and anti-obesity effects (Cho *et al.*, 2012). A multi-targeted, anti-obesity effect of carvacrol - one of the major constituents of caraway - on animals was demonstrated through modifying the gene expressions associated with inflammation and adipogenesis (Cho *et al.*, 2012). Evidence also shows that there is a relationship between the gut flora and obesity (Angelakis *et al.*, 2012; Armougom *et al.*, 2009). Consequently,

plant materials such as caraway, which have intestinal relaxant and soothing effects (Al-Essa *et al.*, 2010) could also possess anti-obesity properties. However, there is no clinical scientific evidence which specifically focusses on exploring the possible role of caraway on weight loss. The aim was therefore to investigate the therapeutic potential of caraway aqueous extract on clinically obese and overweight human subjects.

Despite a significant number of in vitro and in vivo studies on the constituents of caraway and their remedial effects (discussed in the next chapter), there are limited clinical studies on the effects of this plant on weight. Hence, there is a need to examine the anti-obesity effect of caraway clinically. Accordingly, the problem statement of this study will be explained in the next section.

1.1 Problem Statement

Today, weight control is recognized as a common human concern. According to Weiss and colleagues (Weiss *et al.*, 2006), 51% of American adults above 20 years old had tried at some point to control their weight. This subject has attracted the attention of manufacturers, personal health advisors, physicians, patients, and especially governments, to find and develop new approaches and improved solutions for the treatment and prevention of obesity. One attractive method of losing weight is the consumption of natural and synthetic anti-obesity drugs, the long-term usage of these products is typically not under any medical supervision (Blanck *et al.*, 2001). In addition, of the different weight loss pills available in the market, including Xenical (Orlistat), Phentermine/Fentermine, Meridia (Sibutramine), Adipex, Bontril, Didrex, Phentermine, and Tenuate, only two are USFDA-approved: namely Orlistat and Sibutramine (Padwal & Majumdar, 2007; Weigle, 2003). The long-term consumption of the present anti-obesity products is not

recommended as they have exhibited several side effects, including gastrointestinal, psychiatric, and kidney problems which might be irremediable. Such negative symptoms may be due to changes in metabolic rate, and the metabolism of dietary intake (Blanck *et al.*, 2007; Rucker *et al.*, 2007).

Another important issue in the application of such dietary products is their efficacy, short-term and long-term. Some of these supplements might be effective only if taken along with a suitably modified weight loss diet and enhanced physical activity. Consequently, these remedies may be useful only over a short period of time, as the body usually adjusts quickly to most of these dietary supplements. These negative trends may misguide patients, wherein the products do not satisfactorily provide a long term impact on weight loss, and are not tolerated on a chronic basis (Pittler & Ernst, 2004). In this regard, the permitted promotional claims on dietary and slimming products sold to enhance weight loss are relevant, since they pertain to patient expectations without a clinical evidence base.

The current methods being used for the treatment of obesity, such as synthetic anti-obesity drugs, various dietary supplements, or bariatric/gastric bypass surgery are not satisfactory for addressing the issue of obesity on a long-term, global basis because of high consumer cost, limitations of chronic usage, and unfavourable side-effects (Balsiger *et al.*, 2000). Therefore, obesity remains a major global health challenge, and accessible solutions for sustainable weight loss and prevention of weight gain are urgently needed (Fouad *et al.*, 2006). There is a profound lack of scientific information on the rationale for using the presently available alternative therapies, such as dietary supplements, anti-obesity drugs, and other slimming products. Hence, patients are confused in deciding between synthetic

weight loss pills and slimming aids on one hand, and natural sources, such as medicinal plant products, on the other hand. Patients are therefore challenged in searching for a safe and effective method of long-term weight management.

However, despite the strong global market influence, and patient desperation for alternative anti-obesity products and traditional medicinal plants, the awareness of the usefulness of these products is neither sufficient nor clearly perceived. In major part, this is because there is still doubt about their quality, standardization, safety, and efficacy for long-term human use (Kumari *et al.*, 2011; Smyth & Heron, 2006). So, it is necessary to seek for other sustainable solutions and examine other potent natural sources for treating obesity. From that point of view, the researcher aims to introduce one of the potent traditional medicinal plants to be used as a natural weight loss adjuvant. In so doing, this study will examine the effect of CWE on body weight loss.

1.2 Contribution and Significance of the Study

The context of this study is that the use of natural remedies for inducing weight loss has increased dramatically over the last few decades, and typically involve the inclusion of particular medicinal plants in the diet on a regular basis to assist an individual to lose weight gradually (Chang, 2000). Most of the anti-obesity medications studied presently are based on plants used in traditional medicine, as they have been found to be more acceptable than the synthetic medications (Kumari *et al.*, 2011). One example is the weight loss reported in animals and humans treated with "WeighLevel", a combination of four medicinal plants used in traditional Arabic and Islamic medicine, including the leaves of Alchemilla vulgaris, Olea europaea, and Mentha longifolia, the seed extract of Cuminum cyminum, and other ingredients (Said *et al.*, 2011).

In traditional medicine resources, caraway is recommended as a remedy for a variety of health problems, especially digestive disorders (Sadowska & Obidoska, 2003). Moreover, based on a patented natural supplement formula, combinations of carminative herbs, including caraway, have been used to reduce the adverse effects of weight loss drugs, such as orlistat and oral lipase inhibitors (Thompson, 2008). Caraway seed acts as a carminative, and adding this herb to the diet helps in preventing or relieving flatulence. The carminative volatile oils present in caraway induce a relaxant effect on the movements of the intestine muscle (Alhaider *et al.*, 2006; Charles, 2013; Plant & Miller, 1926). Such an effect will synergistically aid in digestion, which, in turn, has a direct effect on food absorption and calorie intake. In addition, using this spice will provide healthful and therapeutic effects for the patient, and will improve the taste and flavor of the final product (Mariaca *et al.*, 1997). Hence, adding caraway to the recipe of food products, may lead food technologists towards novel formulations in the production of functional food preparations.

1.3 Objectives of the Study

This study will present information on the properties of caraway as one of the traditional medicinal plants, and to determine the effect of the consumption of CWE in the management of obesity and overweight in human. To fulfil this aim, the following objectives are determined for this study:

1. To propose the effect of CWE intake on body composition, anthropometric indices and appetite in overweight and obese women

2. To disclose the safety of CWE intake for human

3. To identify the types of phytochemicals present in CWE

1.4 Research Methodology

In order to achieve these objectives, two different methods were applied. A randomized triple-blind placebo-controlled clinical trial was planned to examine the efficacy and safety of caraway intake on overweight and obese women in Yazd, Iran. Also, the chemical analysis of CWE phytochemicals were done using Gas chromatography-mass spectrometry (GC-MS) technique.

1.5 Limitations and Scope of the Study

As mentioned before, based on the main concerns and priorities of WHO traditional medicine strategies on the both safety and efficacy issues for examining traditional medicinal plants, this study has only focused on the safety and efficacy of caraway water extract as a potential traditional medicinal plant for weight loss.

Respondents in this study were selected from healthy overweight and obese women aged 20-55. CWE is consumed regularly in Iran especially in Yazd for losing weight. Furthermore, as the rate of obesity is mostly higher in females, and women are usually more interested to attend weight loss programs than men (Yach *et al.*, 2006), the researcher have selected only overweight and obese women as the study population. Among the recruited candidates, only healthy women with BMI of more than 25 were included and screened for the dietary intervention program. Pregnant and lactating women and individuals who suffered from specific health problems were excluded from this intervention. In general, individuals presenting with any medical condition or the use of

any medication that could have interfered with the conduct of the study or placed the prospective subject at risk; or known allergy or sensitivity to any of the ‘active’ or ‘placebo’ product ingredients were excluded.

Furthermore, the population is selected from Yazd wherein caraway is the common medicinal herb which is used traditionally in their regular diet as a flavoring for culinary purposes and also of its remedial benefits especially for decreasing weight in the form of water extract. Hence, it could be more acceptable for the population and also it would be easier to convince the individual to participate in this dietary intervention program. Moreover, caraway water extract is an affordable product which would be easily accessible at the Yazd market in the low price for consumption.

The required data will be collected through questionnaire, face-to-face interview, physical examination, and biochemical tests on the candidates. In general, assessments were on body composition, anthropometric indices, appetite, and clinical and para-clinical variables of participants during twelve weeks intervention period. As there are a number of studies on anti-obesity effect of caraway constituents on animals, here, the researcher will conduct a clinical trial to evaluate the effect of CWE on human body weight.

This study will introduce an alternative, natural product-based approach for weight loss which is potentially cheaper and healthier to consume, and with minimum human health risks. It is hoped that the results of this research will lead to additional studies which eventually will help patients shift from a temporary weight loss solution to a dietary practice that is long-lasting and sustainable. The findings of this study may be a useful indicator for patients who are not satisfied with the currently available slimming products,

and are still seeking a suitable, safe, and natural alternative. Incorporating natural products with potent anti-obesity properties into a daily human dietary regimen could be a safe, effective, consistent, and inexpensive method for both the treatment and prevention of obesity.

CHAPTER 2: LITERATURE REVIEW

2.1 Traditional and Complementary Medicine

2.1.1 Background

Traditional and Complementary Medicine (T&CM) is a form of health-related practice with a long history in disease prevention, treatment, and management of different diseases especially for chronic ailments. Based on a recent WHO report on "Traditional Medicine (TM) Strategy 2014–2023", "traditional medicine (TM) is an important and often underestimated part of health services". In a number of countries, Traditional Medicine (TM) or non-conventional medicine is also characterized as complementary medicine (CM) (World Health Organization, 2014b). As stated by WHO (World Health Organization, 2001), "Traditional medicine includes a diversity of health practices, approaches, knowledge, and beliefs incorporating plant, animal, and/or mineral-based medicines; spiritual therapies; manual techniques; and exercises, applied singly or in combination to maintain well-being, as well as to treat, diagnose, or prevent illness."

According to a report by the National Medical Advisory Committee, Scottish Office Department of Health, Complementary Medicine and the National Health Services (1996), "Complementary medicine, in practice refers to a wide range of health interventions originating from different cultures across thousands of years of history." In fact, TM is an ancient therapeutic practice which evolved and was practiced in human societies before the application of modern medical sciences to health care. It has developed based on various historical backgrounds and traditional ethnic origins (World Health Organization, 2009b). Examples of T&CM practices include Iranian traditional medicine, Malay traditional medicine, many African traditional medicine systems, Islamic medical practice,

traditional Chinese medicine (TCM), traditional Indian medicine systems, such as the Unani and Ayurvedic systems, and many local practices of indigenous groups in other parts of the world (Shamsuddin, 2011). Complementary and Alternative Medicine (CAM) has been extensively and globally applied for decades. Although the use of modern medicine is widespread, the practice of TM is still applied in most countries of the world. However, application of TM is frequently not involved as a part of the accepted medical system by the government, and it is one of the numerous forms of non-standardized health-care options (Bodeker *et al.*, 2005).

Traditional and complementary medicine plays a crucial role in the aspect of prevention, health promotion and healing. In some countries, it is used to quicken the remedial procedure, and also in sustaining health after treatment (World Health Organization, 2009b). At present, T&CM is a significant part of the health-care system which co-exists with modern medicine to improve health and the quality of life (Azaizeh *et al.*, 2010). In a number of Asian and African nations, 80% of the people rely on TM for primary health care issues. In various developed countries including Australia, the United States, and European countries, 70% to 80% of the people practice some form of T&CM, especially using traditional medicinal plants (Barnes *et al.*, 2008; Shamsuddin, 2011). At present, more than 100 million (one fifth) Europeans are using T&CM on a regular basis, and showing a higher preference for the T&CM system (European Information Centre on Complementary and Alternative Medicine - EICCAM, 2013).

2.1.2 Policies on Traditional Medicine and Regulation of Herbal Medicines

Traditional medicine has continuously sustained its global popularity. During the recent decades, the practices and applications of CAM has been growing in several developed

and developing countries. Numerous TM practices are developed in various nations and regions with different cultures. Over 70% of the people living in developing countries still depend mainly on the CAM practices (Azaizeh *et al.*, 2010). The safety, efficacy, and quality control of TM and CAM have become significant concerns for both the health-care system and the patients. However, there is no comparable improvement of transnational and global standards and applicable approaches or appropriate systems for assessing TM safety, efficacy, and quality control (World Health Organization, 2005).

According to the latest report of WHO on TM (World Health Organization, 2014b), different countries have encountered a number of problems concerning regulatory issues associated with application of T&CM. These issues include lack of research data and knowledge about medicinal plants; lack of appropriate systems to monitor and regulate herbal medicines, and T&CM advertising and claims; a deficiency of suitable assessment techniques to evaluate the safety of these products, and to control and regulate T&CM providers; insufficient financial support for the study of T&CM, a lack of proficiency and expertise within national health organizations and control agencies, insufficient education and training of providers, and an absence of cooperative channels between national health organizations to share data and knowledge about T&CM. These factors are sometimes responsible for the delays in the formation or updating of national strategies, laws, and protocols for TM, CAM, and herbal medicines. To meet these challenges, the WHO TM Strategy was established "to support countries in:

a) Harnessing the potential contribution of TM to health, wellness and people centred health care

b) Promoting the safe and effective use of TM by regulating, researching and integrating TM products, practitioners and practice into health systems, where appropriate
c) Developing proactive policies and implementing action plans that will strengthen the role TM plays in keeping populations healthy."

The WHO 2014 report was a follow-up to the earlier WHO Traditional Medicine Strategy 2002–2005 (World Health Organization, 2002) which had aimed to establish four main objectives:

a) "Policy — integrate TM within national health care systems, where feasible, by developing and implementing national TM policies and programs.
b) Safety, efficacy and quality — promote the safety, efficacy and quality of TM by expanding the knowledge base, and providing guidance on regulatory and quality assurance standards.
c) Access — increase the availability and affordability of TM, with an emphasis on access for poor populations.
d) Rational use — promote therapeutically sound use of appropriate TM by practitioners and consumers."

This new strategy document necessitates Member countries to define and regulate their own countrywide conditions with regard to T&CM, and then to improve and implement guidelines, policies, strategies, and protocols that reveal these authenticities. Member countries are suggested to respond to these challenges by establishing and organizing their accomplishments in the subsequent three strategic directions and strategic actions listed as follows:

"1. Build the knowledge base that will allow T&CM to be managed actively through appropriate national policies that understand and recognize the role and potential of T&CM.

2. Strengthen the quality assurance, safety, proper use and effectiveness of T&CM by regulating products, practices and practitioners through T&CM education and training, skills development, services and therapies.

3. Promote universal health coverage by integrating T&CM services into health service delivery and self-health care by capitalizing on their potential contribution to improve health services and health outcomes, and by ensuring users are able to make informed choices about self-health care."

Although important improvements were made known in applying these strategies around the world, Member states continued to encounter challenges associated with:

a) "Development and enforcement of policy and regulations;

b) Integration, in particular identifying and evaluating strategies and criteria for integrating TM into national and primary health care (PHC);

c) Safety and quality, notably assessment of products and services, qualification of practitioners, methodology and criteria for evaluating efficacy;

d) Ability to control and regulate TM and CM (T&CM) advertising and claims;

e) Research and development;

f) Education and training of T&CM practitioners;

g) Information and communication, such as sharing information about policies, regulations, service profiles and research data, or obtaining reliable objective information resources for consumers."

In general, countries encounter important challenges in the application and development of the T&CM and herbal medicines regulation. These challenges are linked to the regulatory conditions, safety and efficacy valuation, quality control, safety checking, and an absence of awareness about TM/CAM within the nation-wide drug regulatory system.

2.1.3 Challenges Associated with the Regulatory Status of Herbal Medicines

Before synthetic drugs became common, the application of medicinal plants played a significant, in many instances sole, role in treating different ailments (Roberti di Sarsina, 2007). Different countries have great dissimilarity in the description and classification of medicinal plants (Association of the European Self-Medication Industry-AESGP, 2010). A medicinal plant might be known as a food, a functional food, a dietary supplement, a phytotherapeutical, or a herbal medicine being contingent to the protocols relating to the regulations applied to foods and drugs in each country. This issue causes difficulty in establishing what information should be available on medicinal plants for national drug instruction in training centres, and also patients might become confused of using these products (World Health Organization, 2005).

2.1.4 Challenges Associated with the Medicinal Plants

There are several issues and complications associated with the use of herbal medicines. The most important issues are including the evaluation of quality, safety, and efficacy

monitoring of medicinal plants in a national strategy (World Health Organization, 2000a). In general, procedures and necessities for investigation and assessment of the quality, safety and efficacy of medicinal plants are more challenging than those for orthodox medicines and drugs (World Health Organization, 2010). A single medicinal plant is comprised of numerous phytochemicals, and a mixed medicinal plant preparation may have hundreds of constituents. Moreover, excessive time, means and resources are needed to isolate every bioactive constituent from every plant. In reality, such an examination and analysis is practically unmanageable and difficult, especially for the mixed medicinal plant preparations (World Health Organization, 1998a).

Moreover, the safety and efficacy of medicinal plants is determined by the quality of the ingredients applied in their production. Also, the quality of ingredients is closely linked with intrinsic aspects (genetic) and extrinsic aspects (environment, growing crop and harvesting conditions, collecting field and post-harvest, transportation and storing) (Fong, 2002). Consequently, it is very problematic and hard to accomplish quality controls on the basic and primary resources of medicinal plants (World Health Organization, 2011b). In the quality control of final medicinal plant preparations, especially a mixed herbal formula, it is harder to determine the safety and efficacy of the mixed product. Because the combination of phytochemicals might result in inter-reactions between the ingredients which also probably affects the quality of the mixed product (Heber, 2003; World Health Organization, 1998c). Adverse effects caused by use of medicinal plants might be due to several reasons. These factors involve the consumption of the mistaken species of herb, contamination or adulteration of plant product, over dosage, misusage, or drug interactions. Consequently, the investigation of adverse or side effects associated with the consumption of medicinal plants is more problematic and complicated than modern

medications (World Health Organization, 2004). Additionally, medicinal plants are usually used for self-care; hence, there is a substantial need to educate patients and community in their appropriate and correct use (World Health Organization, 1998b, 2009a).

Overall, the policy could play important role in regulating safety and efficacy of traditional medicinal plants which is considered as a challenging issue in recent researches. Herbal medicine is generally categorized into four elementary classifications including: "Traditional Chinese herbalism, Ayurvedic herbalism, Western herbalism, and Traditional Islamic herbal medicine" (Azaizeh *et al.,* 2010). One of the options is traditional herbal medicine based on Islamic perspectives which will be discussed in the next section.

2.1.5 Traditional Medicine and Practices in Iran

The practice and research on medicine in Iran goes back to the olden times over six centuries ago. TM in Iran reached to its uppermost activities during nine and tenth century AD. However, it started to weaken at the beginning of 18th century mostly due to the development of allopathic medicine. Regardless of the growing expansion of the allopathic medical structure, TM retained its popularity with patients. The endurance and sustainability of TM among Iranians are mostly due to public belief and more trust in TM, the failure of orthodox medicine in curing certain ailments, the research and practice on medicinal plants among the patients and scholars, and Islamic perspectives (Bodeker *et al.*, 2005; Mosaddegh & Naghibi, 2001).

Islamic medical practices were likely implemented from the Byzantine and Persian philosophies. Ancient Iranian Medicine called "Irani-tebb/Tebb-e-Sonnati" is one of the

well-known forms of TM which was developed by Ibn Sina, known as Avicenna in the West (Peewãz, 1986; wikipedia.unicefuganda.org, 2013). The most influential medical texts are from the medieval Persian Islamic Medicine in the 9th and 10th centuries AD. Some of these references are mentioned in **Table 2.1** (Ghadiri & Gorji, 2004). One of the greatest methodical and inclusive manuscripts was Avicenna's Canon of Medicine, which was translated into Latin and then distributed all over Europe. This medical literature was used in European scientific centres for more than 600 years and only during the period of the 15th and 16th centuries, it was published above 35 times (Siraisi, 2001).

The Islamic Republic of Iran established its national policy on TM/CAM in 1996, along with developing guidelines and regulations on herbal medicine. Annual market sales in Iran for herbal products was around US$ 3 million, in 1999, US$ 3.1 million in 2000, and US$ 3.5 million in 2001 respectively (World Health Organization, 2005). In recent two decades, there is great rise in experimental studies on Iranian TM using modern scientific methods (Bodeker *et al.*, 2005). These studies raised the possibility of revival of traditional treatments on the basis of evidence-based medicine (Gorji & Khaleghi Ghadiri, 2001).

One of the well-known medicinal plants in Iranian traditional medicine, is caraway with several healing properties. It is mostly used for alimentary problems due to its carminative and stomach-calming properties. In Iranian-Islamic traditional references such as Makhzan Al-Advieh, regular consumption of caraway extract is prescribed for losing weight (Aqili Khorasani, 2001), and today, it is sold as an anti-obesity product in Iran’s markets. In this study, the researcher will examine the anti-obesity effect of caraway on human through a randomized clinical trial (RCT).

Table 2.1: Some of the most influential medical texts in Islamic medicine

Persian Name	Name in Farsi	English/Latin Name/Meaning	Author	Theme, description	Century
Firdous al-Hikmah	فردوس الحكمة	Paradise of Wisdom	Al-Tabari (teacher of Rhazes)	Ancient medical encyclopedia on general medicine, psychotherapy, …	9 (A.D)
Kitab al Nibat	كتاب النبات	Book of Plants	Ibn Dawoud Al-Dinawari (the father of Arabic botany)	Described more than 600 plants and their uses in his book	9 (A.D)
al-Hāwī fī al-Tibb	الحاوي فى الطب	TheVirtuous Life, Liber Continens	Rhazes, Rāzi	The comprehensive book on general medicine	9 (A.D)
Al Mansuri Fi al-Tibb	الكتاب المنصوري في الطب	Liber Medicinalis ad. Almansorem,The Book on Medicine Dedicated to al-Mansur	Rhazes, Rāzi	General medicine, medical pathologies of the body	10 (A.D)
al-Judari wa al-Hasbah	الجودرى و الحصبه	Treatise on small pox and measles	Rhazes, Rāzi	Infectious diseases, differential diagnosis	10 (A.D)
Al-Qanun fi'al-Tibb	القانون في الطب	The Canon, the rules of medicine	Avicenna	General medicine	10 (A.D)
Resaleh dar Nabz	رساله در نبض	Pulse	Avicenna	Cardiovascular diseases	10 (A.D)
Zhakhireh Kharazmshahi	ذخيره خوارزمشاهى	The treasure of Khvarazm'Shah	Esmail Jorjani	General medicine	10 (A.D)
Somom	سموم	Poisons	Qhortabi	Toxicology	10 (A.D)

Source: Modified from Ghadiri and Gorji (2004)

In conclusion, based on previous studies, one of the recent initiatives of the Western Pacific Regional Office of WHO has been the development of a revised Regional Strategy for Traditional Medicine in the Western Pacific for the period 2011-2020 (World Health Organization, 2012). The strategic directions describe possible approaches to improve the quality, safety, and efficacy of traditional medicinal plants in health care in the region, and recognize that the 27 regional countries embrace broad stages of economic development and research capacity. Cooperation within and between countries to address issues of quality, safety, and efficacy of botanical materials of known or standardized content is

therefore strongly encouraged to enhance regional health care (World Health Organization, 2014b).

Since obesity is recognized as one of the global health concern, and also recently, there has been a renewed interest in natural obesity medications and application of herbal medicine for weight loss, in the next section, the researcher will discuss on obesity as a challenging health issue, describing the prevalence, risk factors, and consequences of obesity, coming together with the challenges in treating obesity. Further, the weight loss claims on dietary supplements and natural medications will be discussed, focusing on the safety and efficacy of medicinal plants. Finally, the researcher will give details on the application of caraway, as a potent traditional medicinal plant for weight loss. According to the ancient Persian-Islamic references in the 9th and 10th century AD such as Rhazes' book al-Hāwī fī al-Tibb, Ibn Dawoud Dinawari's book Kitab al Nibat (Book of Plants) and Avicenna's Al-Qanun fi'al-Tibb (The Rules of Medicine), caraway is acclaimed to have healing properties especially for treating obesity. Hence, the researcher has tried to conduct a clinical trial to evaluate the efficacy and safety of caraway water extract on overweight and obese women.

2.2 Obesity and Overweight

Obesity is an important global health concern, and is associated with high morbidity and mortality rates. Today, it is recognized as a global health problem which occurs as a result of the accumulation of excess fat in the body. Based on the classification by WHO (World Health Organization, 2000b) a body mass index (BMI) greater than 25 kg/m^2 is defined as overweight (pre-obese) and BMI value of greater than 30 kg/m^2 is termed as obesity. This classification is shown in **Table 2.2**. Despite a variety of studies on the

treatment and management of this disease, "Globesity" remains a challenging issue (Cheng, 2006). Most interventions conducted on obese children afforded outcomes which were negligible, demonstrating the requirement for more specific, focused research (Boon & Clydesdale, 2005). The majority of the current approaches to treat obesity typically use synthetic chemical-based medicines. However, the high costs and the side-effects of these drugs necessitate that patients and researchers in most of the world seek alternative therapeutic approaches (Rucker *et al.*, 2007). One of the main factors related to prevalence, as well as the management of obesity, is the dramatic changes that have occurred on a global basis in dietary patterns during recent decades (World Health Organization, 2003).

Table 2.2: Classification of obesity according to WHO

BMI	Classification
< 18.5	underweight
18.5–24.9	normal weight
25.0–29.9	overweight
30.0–34.9	class I obesity
35.0–39.9	class II (severe obesity)
≥ 40.0	class III (morbid obesity)

Source: World Health Organization (2000b)

2.2.1 Prevalence of Obesity

Since 1997, the World Health Organization (WHO) has warned of obesity as a global epidemic although it was not noticeable during most of the 20th century. Statistics show that the prevalence of obesity had reached 400 million adults by 2005, which is equal to 9.8% of the global population, and had risen to 500 million (11%) in 2008. Also, 1.4 billion equal to 35% of adults over 20 years old were overweight in 2008. Recently, WHO has informed that global prevalence of obesity and overweight is almost doubled since 1980 (Caballero, 2007; World Health Organization, 2011a).

As the population grows, simultaneously, obesity penetrates all layers of the population. Across OECD (Organization for Economic Co-operation and Development) countries, one in two adults is currently overweight, and one in six is obese (Maury & Brichard, 2010). The rate of overweight people (BMI>25 kg/m^2) is projected to increase by a further 1% per year for the next 10 years in some countries (Sassi, 2009). **Figure 2.1** shows this trend graphically. This prevalence is greater in older people, especially in the age range of 50-60 years old, and higher rates of obesity are seen among women than men (James, 2008; Seidell, 2005). Besides, severe obesity grows faster than the overall rate of obesity in the developed and high income countries such as United States, Australia, and Canada (Howard *et al.*, 2008).

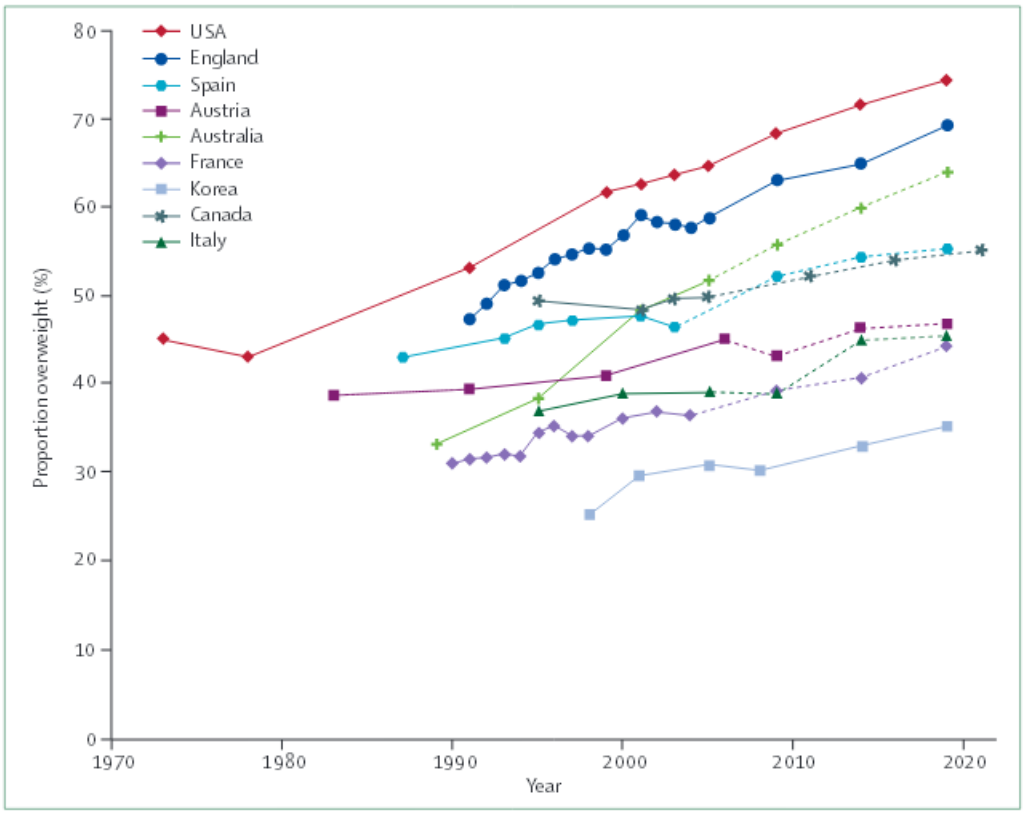


Figure 2.1: Past and projected overweight trends in different countries

Adopted from: Sassi (2009)

According to the 'International Association for the Study of Obesity/International Obesity Taskforce' (IASO/IOTF) analysis (2010), around 1.0 billion adults are presently overweight, and an additional 600 million are considered obese globally. Also, up to 150 million school aged children are overweight, and 40-50 million are categorized as obese. In the European Union (EU) 27 member states, almost 260 million (60% of adults) and over 12 million equal to 20% of children are either overweight or obese. In addition, severe obesity (BMI between 35-39.9) has grown faster than the overall rate of obesity in the developed countries, such as the United States, Australia, and Canada (Howard *et al.*, 2008). However, during recent decades, obesity has penetrated the developing countries, even in the rural areas, at a faster rate (Kopelman, 2000; World Health Organization, 2014a). Sub-Saharan Africa is the only area where obesity is not seen to any great extent (Haslam *et al.*, 2006). In Western Africa, obesity rates in 2008 were around 10% which was higher in urban residents and women (Abubakari *et al.*, 2008). In China, overweight and obesity in adult raised from 12.9% in 1991 to 27.3% in 2004 (Popkin, 2007). The overweight and obesity rate in Iran in 2008 was 40.6% and 26.3% respectively, wherein, the rate of central obesity was higher among women (72.2%) than men (26.6%) (Rashidy-Pour *et al.*, 2009). In 2013, the prevalence of overweight and obesity in the adult Iranian population over 20 years old has been raised up to 50 to 70% (Delshad, 2013). In brief, obesity has become a widespread disease existing in different social, economical, cultural, regional, and age groups. Consequently, this hidden hazard is affecting human life multi-dimensionally (Kottke *et al.*, 2003; Yach *et al.*, 2006). The next section will discuss on the obesity causes and risk factors.

2.2.2 Etiology and Risk Factors of Obesity

Urbanization and modernization are reported as two major factors related with obesity. Following globalization, people in the developing countries are also accepting the unhealthy Western dietary habits and lifestyles which contribute to weight gain and obesity. During recent decades, intake of sweetened beverages, vegetable oils and animal-origin foods (meat, poultry, fish, eggs and dairy products) is increased dramatically especially in low income population and developing countries (Radzi *et al.*, 2013). Although governments are bearing in mind various interventions and aid programs, still limited countries have been able to effectively reduce their overweight populations (Popkin, 2004; Popkin, 2007). Nutritionists believe that sedentary lifestyles, stress, and dietary habits resembling those of Western countries are the main underlying factors of overweight and obesity in the global population (Maddock, 2004; World Health Organization, 2011a).

2.2.3 Obesity Consequences and Health Problems

Overweight, as a major obstacle in the maintenance of human health, may lead to a large number of chronic diseases (Wyatt *et al.*, 2006). Moreover, the consequences of obesity in terms of morbidity and mortality are very important, as it is now one of the leading causes of death worldwide. According to the classification of obesity by WHO, a body mass index (BMI= weight/height^2) value of greater than 40 Kg/m^2 is termed "morbid obesity" (World Health Organization, 2000b). Studies show that obesity is related to cardiovascular diseases, hypertension, diabetes mellitus, gallbladder disease, different types of cancer, endocrine and metabolic disturbances, osteoarthritis, gout, pulmonary

diseases, as well as psychological issues, including social bias, prejudice, discrimination, and overeating (Mokdad *et al.*, 2004; World Health Organization, 2011a).

Excess body fat is also associated with the development of several life-threatening chronic conditions (Guh *et al.*, 2009). According to a WHO report, obesity is related to a wide range of health problems, including cardiovascular diseases, hypertension, diabetes mellitus, gall bladder disease, cancer, endocrine and metabolic disturbances, osteoarthritis, gout, pulmonary diseases, and eating disorders, as well as psychological problems resulting from social bias, prejudice, and discrimination (World Health Organization, 2011a). Accordingly, overweight is regarded both as a cosmetic issue, and as a major risk factor for human health (Kopelman, 2000) which may decrease life expectancy (Olshansky *et al.*, 2005). Consequently, the potential health benefits of a reduction in excess weight are of considerable public health and economic importance (Sofi *et al.*, 2010). However, despite vast attempts to address this issue, "globesity" remains an enormous challenge.

From an economic perspective, obesity, and its related health consequences, involves enormous costs for both the current and future health care systems, including physician visits, hospitalization, and numerous other related expenses (Picot *et al.*, 2009; Wolf & Colditz, 1998). According to recent statistics, the health care costs related to obesity and its complications in 2005 were estimated around $70 to $100 billion, and this amount is projected to increase quickly with rapid escalations in the prevalence of obesity and its health complications such as diabetes, heart diseases, and cancer, which is unavoidable. These data include Medicare, Medicaid and private insurers, and reflect the ascending trend in the obese adult population in the US from 12% to 35.7% between the years 1989-2010, and which is projected to rise further to 47.5% by 2020. In short, obesity can cause

a decline in life expectancy (Olshansky *et al.*, 2005). However, despite vast attempts to address this issue, "globesity" remains an enormous challenge (Delpeuch *et al.*, 2013; Wang *et al.*, 2011). Based on the previous literature review, there are still challenges in the current methods for treatment and management of obesity which will be discussed in the next section.

2.2.4 Challenges in Treating Obesity

Today, weight control is recognized as a common global concern (Kruger *et al.*, 2004). Millions of people have attempted to control their weight at some time to have a healthier and happier life. One of the most favoured ways for losing weight is self-medication with over-the-counter anti-obesity pills and weight-loss dietary supplements. According to a survey of around 15,000 US adults, in 1996-1998, 7% of them had consumed non-prescription weight loss products. It is presumed that this percentage would be greater if a survey were conducted today. Also, the demand for these slimming aids is high, and it is likely to increase simultaneously with obesity rates (Blanck *et al.*, 2001). During 2000-2001, Americans spent $50 billion each year on anti-obesity programs and products. However, $6 billion of this was spent on dietary supplements and weight loss pills. This investment in personal concerns with respect to weight rose to $55.4 billion in 2006 (Blanck *et al.*, 2007; Sharpe *et al.*, 2007). However, most of the long-term usage of these weight-aid products is not under any medical or nutritional supervision (Kruger *et al.*, 2004).

2.2.5 Weight Loss Claims on Dietary Supplements

Recently, there has been a proliferation of different anti-obesity products appearing on the market (Viner *et al.*, 2009). They are typically high-cost products and their long-term consumption is not recommended, as several have exhibited significant side effects, including gastrointestinal symptoms, kidney and liver dysfunctions, and even psychological problems (Rucker *et al.*, 2007). For example, among the varieties of anti-obesity drugs, only *Orlistat* and *Sibutramine* can be used long-term (Padwal & Majumdar, 2007). In addition, a number of the synthetic products and dietary supplements do not satisfactorily impact weight loss, or are not well-tolerated (Dwyer *et al.*, 2005). Meanwhile, the use of natural remedies for weight loss has increased. Scientists believe that botanical sources maybe safer, more reliable, and also cheaper than current conventional methods, such as synthetic drugs or surgical procedures, which may have adverse effects, or be of limited duration in effectiveness (Chang, 2000; Clegg *et al.*, 2003; Sui *et al.*, 2012).

Can medicinal plants serve as a sustainable resource for standardized agents which can meet patient expectations for weight loss and provide long-term, consistent health benefits? This issue will be discussed in the following section.

2.2.6 Natural Anti-Obesity Medications: Medicinal Plants

Currently, nutritionists and healthcare specialists believe that a reasonable level of physical activity, in combination with proper eating habits, are the two basic principles of a healthy lifestyle (Lau *et al.*, 2007). Habituating these major components will help individuals to maintain their body weight and health in the ideal situation. Meanwhile,

approaching a balanced body weight could be easier and faster by integrating other useful methods which enhance weight loss. One of these methods is the consumption of medicinal plants which people use traditionally to lose weight. Recently, the use of natural remedies for weight loss has increased, based on reliability, safety, and cost compared with synthetic drugs or surgical procedures, which may have limitations. Incorporating these natural ingredients within a daily diet will assist an individual to lose weight in a cheaper, easier, faster, and healthier way (Picot *et al.*, 2009; Roy & Nallanayagam, 2011). Here the researcher will provide a review of the accessible botanical sources for the treatment of obesity, explaining how these medicinal plants act in humans to cause weight loss, and which method of usage is safer and more efficient.

Studies show that a number of natural food ingredients and medicinal plant preparations are able to enhance satiety, boost metabolism, and speed up weight loss (Yun, 2010) . The weight lowering effects of these potent plants might be due to the presence of fibre which suppresses appetite, or specific phytochemicals such as caffeine, phenolic compounds, etc. which are able to down-regulate obesity by enhancing thermogenesis, lipolysis, etc. (Hsu & Yen, 2008; McCrory *et al.*, 2010). Including these foods in the diet on a regular basis will therefore assist an individual to lose weight slowly and safely. However, there are still some doubts about the application of these products for humans. Because usually, there is a misunderstanding in the consumption of plant products without any limitation in dosage, preparation and composition (mixed with other herbs) while in some cases, consumption of a number of plant-based food supplements have shown adverse effects on the human body, such as liver toxicity which might be due to over dosage intake or possible synergistic effects of mixed plant components (Arbo *et al.*, 2009; Dara *et al.*, 2008; Hansen *et al.*, 2013). On the other hand, despite the global market for satiety, fat burning,

dietary supplements, and other weight management remedies, the awareness of the usefulness of these products is neither sufficient, nor clearly perceived by patients, physicians or nutritionists (Esmaillzadeh & Azadbakht, 2008).

2.2.6.1 Mechanism of Action of Antiobesity Medicinal Plants

Natural anti-obesity preparations can induce weight loss through several mechanisms. Their functions can be classified into five major categories, as shown in **Table 2.3**.

Table 2.3: Different functions of anti-obesity medicinal plants in humans

Anti-obesity function	Mechanism of action	Anti-obesity preparations from different natural sources
Reducing food intake	Inhibiting appetite and reducing food intake	pine nut (Pasman *et al.*, 2008), pomegranate leaf (Lei *et al.*, 2007), ginseng (Kim *et al.*, 2005), *Hoodia gordonii* (Van Heerden, 2008)
Enhancing thermogenesis	Stimulating sympathetic nervous system and increasing metabolic rate	sea weed (Maeda *et al.*, 2005; Maeda *et al.*, 2007; Maeda *et al.*, 2008), bitter orange (Haaz *et al.*, 2006), red orange (Dallas *et al.*, 2013), sour orange (Preuss *et al.*, 2002), soybean (Ishihara *et al.*, 2003)
Decreasing the absorption of lipids	Inhibiting pancreatic lipase activity	chitosan (Bondiolotti *et al.*, 2007; Jun *et al.*, 2010), levan (Kang *et al.*, 2006), mate tea (Martins *et al.*, 2009), oolong tea (Hsu *et al.*, 2006; Nakai *et al.*, 2005), green tea (Koo & Noh, 2007)
Preventing adipogenesis	Inhibiting adipocyte differentiation	turmeric (Ahn *et al.*, 2010), capsicum (Hsu & Yen, 2007a), palm oil (Uto-Kondo *et al.*, 2009), banana leaf (Bai *et al.*, 2008; Klein *et al.*, 2007), brown algae (Maeda *et al.*, 2006), garlic (Ambati *et al.*, 2009), flaxseed (Tominaga *et al.*, 2009), black soybean (Kim *et al.*, 2007)
Increasing lipolysis and fat oxidation	Stimulating sympathetic nervous system, enhancing lipid metabolism	herb teas (Okuda *et al.*, 2001), cinnamon (Sheng *et al.*, 2008), orange and grapefruit (Dallas *et al.*, 2008), *Nelumbo nucifera* leaves (Ohkoshi *et al.*, 2007)

Source: Taken from several sources

Based on the inhibition of pancreatic lipase activity, the intake of some medicinal plants will prevent the absorption of lipids in the intestine. Consequently, non-absorbed fat will be excreted through oily faeces. Furthermore, certain bioactive components can promote energy expenditure by increasing basic the metabolic rate, which enhances thermogenesis.

This function will help the body to burn additional calories and excess body fat. Through prevention of adipocyte differentiation, medicinal plant consumption will inhibit adipogenesis and the formation of fat cells in adipose tissues. Moreover, based on enhancing lipid metabolism (lipolysis) some medicinal plant products can increase lipolysis through inducing β-oxidation or noradrenaline secretion in fat cells. Other anti-obesity ingredients are able to suppress appetite and induce satiety, which will help individuals to control their appetite. Finally, these different functions of antiobesity medicinal plants will cause a reduction of food and energy intake (Yun, 2010).

2.2.6.2 Efficacy and Safety of Antiobesity Medicinal Plants

Medicinal plant samples can be collected from the whole plant, or from parts of the plant, such as the stem, bark, leaf, flowers, and roots. These materials are then processed into different forms, such as powder or capsules. However, most of the medicinal plants which have shown antiobesity properties were prepared in the form of aqueous or alcoholic extracts. This may be because the decoction, distillation, and infusion procedures can concentrate the constituents responsible for the therapeutic efficacy of the examined herb.

Some components which might inhibit the anti-obesity function of the bioactive compounds might be removed by the extraction procedure. Extraction and partial purification, or the isolation of the active principle(s) could increase the bioavailability of the bioactive constituents in medicinal plant extracts which consequently will enhance the efficacy of medicinal agent in losing weight (Calixto, 2000).

In other studies, scientists have examined the anti-obesity properties of mixtures of medicinal plants. However, in several cases shown in **Table 2.4**, the consumption of

different antiobesity preparations in combination with other plant-based ingredients could produce unexpected side-effects. Based on previous studies, the application of single medicinal plants has not caused any adverse events. On the other hand, the undesired effects on the human body could be due to interactions between the different phytochemical constituents present in the different plants (Heber, 2003).

In summary, consumption of such potent plants regularly, and in optimum dosage, can induce weight loss gradually and naturally while the chemical anti-obesity products usually act faster with severe and considerable changes on the normal body function, and which most probably could result in unfavorable effects on the human body. Hence, the medicinal plants can be considered as a safe alternative for chemical anti-obesity medications (Preuss *et al.*, 2002).

Table 2.4: Safety and efficacy of antiobesity medicinal plant preparations

Medicinal Plant	*Result	Adverse effects	Combination formula	Result	Adverse effects
Kidney bean (*Phaseolus vulgaris*) (Udani *et al.*, 2004)	+ (p=0.07)	Not reported	In combination with green tea extract (Birketvedt, 2009)	inter group differences (+)	Flatulence, soft stool, constipation
Green tea (Camellia sinensis) (Nagao *et al.*, 2007)	+ (p<0.05)	Not reported	In combination with asparagus, black tea, guarana, kidney bean, Garcinia cambogia and chromium yeast (Opala *et al.*, 2006)	no inter-group difference in weight (-)	Gastrointestinal complaints
Rhubarb (rheum) (Jin & Jiao, 1994)	+	Not reported	In combination with ginger, astragulus, red sage, and turmeric, combined with gallic acid (Greenway, Liu, *et al.*, 2006; Roberts *et al.*, 2007)	greater weight gain in intervention group/ (-)	Gastrointestinal, oral, dermatologic, irritation, headache.
Glucomannan fiber (Walsh *et al.*, 1984)	+ (p<0.005)	Not reported	In combination with chitosan, fenugreek, *Gymemna sylvestre*, vitamin C (Woodgate & Conquer, 2003)	inter group differences (+) (p<0.01)	Constipation, headache, indigestion
Garcinia cambogia (Mattes & Bormann, 2000)	+ (p=0.03)	Not reported	In combination with natural caffeine (Rothacker & Waitman, 1997)	No inter group differences (p=0.3)	Not reported
Bitter orange *Citrus aurantium*) (Stohs *et al.*, 2011)	+	Not reported	In combination with pantothenic acid, green tea leaf extract, guarana, white willow bark and ginger root (Greenway, de Jonge-Levitan, *et al.*, 2006)	greater weight gain in intervention group (p<0.04)	Hypertension, musculoskeletal, neurological, migraine, anxiety

* Results indicate the efficacy and intergroup difference
Source: Taken from several sources

On the other hand, single and mixed, anti-obesity medicinal plant preparations can have different effects. In fact, the dietary intake of these medicinal plants in the natural form, when taken singly, can apparently provide a higher degree of safety and efficacy than when mixed medicinal plant preparations are applied. These findings support the recommendation of many health organizations regarding the consumption of natural ingredients on a regular basis. However, there are other factors which can also affect the results of such studies, including the treatment dosage applied, the quality of the botanical preparations, the route of administration, the presence and concentration of various bioactive components, and their respective functions, the experimental methods used, the

study design, the duration of treatment, and the safety and efficacy of the ingested plant. Introducing such potential plants will help patients to use them as an alternative approach along with other alternative treatments, such as acupuncture and hypnotherapy, in the management of obesity (Sui *et al.*, 2012).

Efforts to improve knowledge for patients regarding the consumption of anti-obesity medicinal preparations other than chemical medicines which have become popular, and provide encouragement to overweight and obese patients to consume them at an optimum dosage, along with an enhanced exercise regimen and a healthy diet should be continued. In addition, more chemical, biological, and controlled clinical studies are needed on the effectiveness of standardized, selected medicinal plants, particularly those used as spices and condiments, in ameliorating and treating obesity in humans.

In the next section, the researcher will discuss on caraway as one of the potent medicinal plants for treating obesity, focusing on various aspects of the background of caraway. Among the topics covered here include the use, the definition and classification of caraway, the botanical description and morphology, the etymology, and the geographical distribution, cultivation, and regions of production. Attention is then turned to the ethnopharmacological and therapeutic applications of caraway, the chemical compounds and the biological activities, and the safety and toxicological evaluations of caraway-based products.

2.3 Caraway (*Carum carvi*)

2.3.1 Background Literature on Caraway

Carum carvi, which belongs to the Apiaceae family, is one of the earliest cultivated herbs in Asia, Africa and Europe. Caraway seeds have remained popular as culinary spices and are also overwhelmingly used in traditional therapy since antiquity in diverse geographical areas.

The Apiaceae family is a collection of typically aromatic plants having hollow stems comprised of more than 434 genera and 3,780 species. Among the well-known members of the family are anise, asafoetida, caraway, carrot, celery, coriander, cumin, dill, fennel, parsley, parsnip, and sea holly (Bennett, 2010; Brechbill, 2012; Papini *et al.*, 2007). Caraway is sometimes referred to as meridian fennel, even though another plant, *Foeniculum vulgare* Mil. (Apiaceae) has also been ascribed the name fennel. In Arabic, *C. carvi* is known as "Karawiya". Previous studies have established an association between the moderate consumption of caraway oil/aqueous extract and other caraway-derived preparations with a lower incidence of diabetes, dyslipidemia, hypertension, liver dysfunction, reproductive hormone imbalance, osteoporosis, cancer, and gastrointestinal and inflammatory diseases (Johri, 2011).

2.3.2 Definition and Classification, Botany and Morphology of Caraway

Caraway is defined as the dried ripe fruit of the biennial, usually white-flowered, aromatic Old World herb (*Carum carvi* L., Apiaceae), also known as Persian cumin or meridian fennel, and is one of the ancient cultivated plants of Asia, Africa, and Europe (Khan & Abourashed, 2011). The fruits (also known, erroneously, as "seeds") are used extensively as a mild spice and flavoring for culinary purposes in many cuisines. Caraway

seeds are also widely used in various systems of traditional medicine, and the aromatic constituents have been studied for their health beneficial effects (Perry & Metzger, 1980; Prance & Nesbitt, 2005). The plant resembles a carrot plant with feathery leaves and is slender, branched, and hollow-stemmed, 30-80 cm in height. The dried, brown fruits are hard, crescent-shaped achenes, around 2 mm long, with five pale ridges (Vaughan & Geissler, 2009). Caraway flowers, and caraway ripened and dried fruits are shown in **Figure 2.2** (© Copyright Mel Harte 2010), **Figure 2.3** (© Les Mehrhoff, 2008-2010), and **Figure 2.4** (Discover Life organization), and in sketched form in **Figure 2.5** (http://www.discoverlife.org/ap/copyright.html). The fruits have a pleasant odor, and an aromatic flavor and sharp taste; they are similar to cumin, with which they are sometimes confused (Prance & Nesbitt, 2005). In Middle East countries they are distinguished by their color; caraway is known as black zeereh and cumin seeds known as green zeereh (Ghazanfar, 2011).

Figure 2.2: Caraway plant in flower

Figure 2.3: Caraway plants with ripening fruits

Figure 2.4: Dried caraway fruits (often termed caraway seeds)
Adopted from: http://www.discoverlife.org/ap/copyright.html

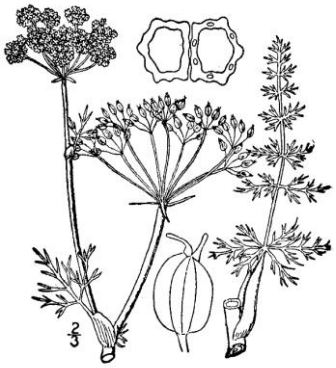


Figure 2.5: *Carum carvi* L.
Adopted from: Britton and Brown (1913)

Several other plants are often mistaken for caraway (*Carum carvi*) due to their similarity in odour or appearance, including anise, fennel, cumin, black cumin, and black caraway (Natural Resources Conservation Service; United States Department of Agriculture: USDA-NRCS, 2013) Please refer to the Appendix A which shows the appearance and common name of some of these aromatic plants. Also, the etymology and synonyms of caraway in different languages will be explained in Appendix B.

2.3.3 Origin, Geographical Distribution, and Cultivation

The origin of caraway, as one of the oldest recognized spice plants, is unknown. However, the names Persian caraway and Roman cumin suggest that it was introduced into global commerce and usage from Persia or Europe. It has been cultivated since olden times, and is found growing wild all over central Asia, especially in Iran, the Himalayas, northern and central Europe, the Balkans, and North Africa. By 1806 it was being cultivated in North America, where it was used as a flavoring to season bread and cheese, and as a pickling spice, to prepare the liquor Kummel, and to give flavor to sausages. Presently, it is mainly cultivated in North Africa, especially Egypt, and in Finland, the Netherlands, Eastern and Northern Europe, and Germany, the Mediterranean regions, Russia, Iran, Indonesia, and North America (Levetin & McMahon, 1999; Rosengarten, 1969; Sadowska & Obidoska, 2003).

Even though the plant is indigenous to Asia, Europe, and Northern Africa, the plants are cultivated in several different regions throughout the world, this plant is better adapted to cooler climates than other Apiaceae species. Caraway plants are grown widely as a winter plant and as a summer crop in different regions, such as in Northern France, and the north Himalayan region in India (Khare, 2008; Prance & Nesbitt, 2005). In warmer

regions, it is cultivated in the winter months as an annual, and in temperate climates it is cultivated as a summer annual or biennial plant. There is still some domestic production of caraway in Europe, including Germany and Scandinavia, even though it is mostly imported from Egypt, and the cultivation of caraway as a spice crop has been expanding in the United States and Canada (Rosengarten, 1969; Sher *et al.*, 2010). **Figure 2.6** shows the point growth map of *Carum carvi* in different regions worldwide.

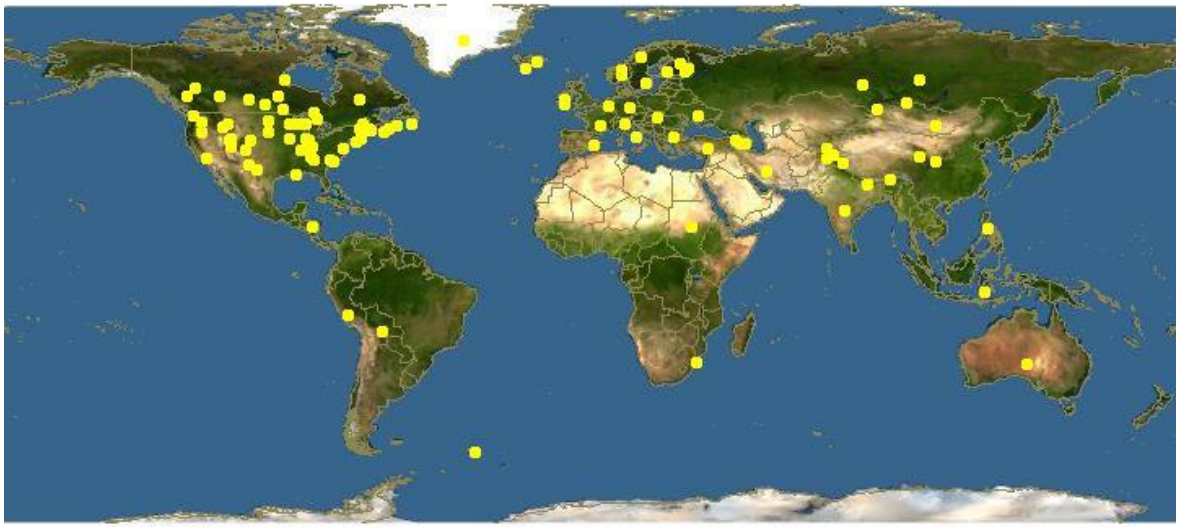


Figure 2.6: Point growth map of *Carum carvi* (Discover Life organization)
Adopted from: http://www.discoverlife.org/ap/copyright.html

2.3.4 Historical Background and Traditional Usage of Caraway

For thousands of years, caraway has been used, particularly in China, Egypt, Roman Britain, the Sumer area, and India, as a culinary seasoning (Jones, 1996), and may have been in use in Europe longer than any other spice. It was used by the Early Greeks in their recipes, and also recommended and prescribed by Dioscorides for healing purposes (Prance & Nesbitt, 2005). It is one of the most important species used as a wild food plant in the Eurasia regions and Estonia (Kalle & Soukand, 2012), and is utilized in the cuisines

of the Middle East, India, Central Europe, and North Africa, especially Tunisia and Yemen (Lim, 2013) for its pungent licorice flavor (Halberstein, 2005), which is derived from the essential oils, especially carvone and limonene. This culinary flavoring is found in sauerkraut, casseroles, curries, and other foods such as specific cheeses, different liqueurs, breads, cakes, desserts, and salads (McKenna, 1999) as well as a spicy marinade for meats, dumplings, and goose (Vaughan & Geissler, 2009). In the Middle East, caraway pudding is a popular dessert during Ramadan, and the seeds are added as a spice in Persian cuisine to foods such as bread, yogurt, pickles, sauces, and salads (Zargari, 1995). Caraway seed oil is also used as a fragrance component in soaps, lotions, and perfumes.

2.3.5 Ethno-Pharmacological and Therapeutic Applications of Caraway in Traditional Medicine

From a therapeutic perspective, caraway is known as a traditional medicinal plant with a long history of healing (Johri, 2011; Jones, 1996; Kenner & Requena, 2001). It is believed to have many of the medicinal properties similar to dill, fennel, and anise, and is reported to have potent anti-spasmodic, antiseptic, aromatic, carminative, digestive, and stimulant properties. It has been used by chewing the raw dried fruits, and also in the form of drink or tea through brewing, decoction, distillation, fermentation, or as a tisane (Bown, 2001; Chevallier, 2000; Chopra *et al.*, 1986; Grieve, 1971; Sastri, 1956). Therapeutic use of caraway products has been widely known in different ethnomedical systems from Northern Europe to the Mediterranean regions, Russia, Iran, Indonesia, and North America, where the use continues as a primary component of traditional treatments (Sadowska & Obidoska, 2003). Traditionally, it was believed to warm and stimulate a cold, languid stomach. In folklore, it is used for the treatment of stomach

complications, dysentery, uterine problems, internal wounds, and ulcers (Fahad & Bano, 2012). The fruits have also been applied in the form of a condiment, and the oil for the treatment of colds, coughs, sore throat, fever, bronchitis, gingivitis, and gastrointestinal complaints (Dachler, 2005).

Greek physicians prescribed caraway oil or seeds for 'pale-faced girls'. Also, Romans consumed caraway to relieve indigestion (Kenner & Requena, 2001). Caraway is well-known for its carminative and stomach-calming properties, being mostly used for alimentary problems (Nasser *et al.*, 2009; Saad & Said, 2011a, 2011b). In the herbal remedies of India and Ayurveda, the fruits of caraway are mostly prescribed as a carminative, eupeptic, anti-spasmodic, and astringent, and applied for the treatment of mild gastrointestinal ailments, such as stomach-ache, bloating, indigestion, cramp, and flatulence due to its stomach-strengthening properties (Balch, 2002; Khare, 2008). Caraway aqueous brewed extract is commonly used for pediatric conditions, especially digestive complaints. Also, in Ayurveda, it is claimed to improve the absorption of other plants, and to promote the function of vital organs, such as the liver. Extracts have also been used in broncho-pulmonary conditions, as a cough therapy, and also as an analgesic. Vapors from caraway seeds are known to provide relief in patients suffering from back pain and rheumatism (Joshi, 2000; Mhaskar *et al.*, 2000; Perry & Metzger, 1980; Sivarajan & Balachandran, 1994). In Tibetan traditional medicine, caraway is considered to have a pungent taste and a warming influence due to its hot nature (Lim, 2013).

In the Moroccan system of folk medicine, the fruits of caraway are well-known as a stimulant, being consumed as a galactagogue to stimulate milk production in lactating mothers, and to stimulate menstruation (emmenagogue) in women, as a digestive and appetite stimulant, also to increase sexual desire (aphrodisiac), and urine flow (diuretic)

(Lahlou *et al.*, 2007). It has been prescribed for healing hyperglycemia, hypertension, also heart and renal diseases (Eddouks *et al.*, 2009; Eddouks *et al.*, 2002; El Amrani *et al.*, 2010; Jouad *et al.*, 2001; Tahraoui *et al.*, 2007). In addition, it has been used to treat flatulent colic in infants, and for relieving stomach complaints, being frequently applied to flavor children's medicines (Bnouham, 2010; Halberstein, 2005; Reynolds, 1996). In Jordanian traditional medicine therapies, caraway is used as a home remedy to treat different gastrointestinal and respiratory problems (Barakat Abu-Rmailah & Afifi, 2000). In the traditional medicine of Poland, caraway is known as a therapy for alimentary disorders, flatulence, appetite imbalances, and as a galactagogue plant, while in Russia, it is recommended as a cure for pneumonia. In Great Britain and the USA, it is considered as a stomachic and carminative. In the Malay Peninsula, caraway is one of the important medicinal plants used during confinement, and in Indonesia, it is regarded as the therapy for inflamed eczema. Also, in India, it has been commonly used traditionally as a female fertility regulating agent (Kumar *et al.*, 2012; Perry & Metzger, 1980; Sadowska & Obidoska, 2003).

In Iranian traditional medicine, caraway has been recommended as an antiseptic, antispasmodic, anti-parasitic, lactigenic, hypolipidemic, anti-flatulence, carminative, and for digestive complains (Mikaili *et al.*, 2011). Also in Iran, caraway is considered energizing, carminative and astringent and its healing properties have been applied to gastrointestinal, gynecological, and respiratory conditions, and recommended for the treatment of toothache, diarrhea, and epilepsy (Ghazanfar, 2011; Zargari, 1995). Today, in the Middle-East region, it is considered a medicinal plant commonly used for losing weight due to its ability to soothe the stomach, improve digestion, and regulate appetite (Dasgupta & Hammett-Stabler, 2011; Saad *et al.*, 2008; Saad & Said, 2011a).

2.3.6 Chemical Compounds of Caraway

A large number of experimental studies investigating the chemical and biological properties of various caraway preparations have been reported attempting to correlate the chemistry and the biological activities of this plant. From a chemical perspective, the aqueous and oil extracts of the roots of caraway have afforded a variety of phenolic and aromatic compounds including different flavonoids, iso-flavonoids, flavonoid glycosides, mono-terpenoids, such as carvone and its derivatives, glucosides, lignins and alkaloids, as well as poly-acetylenic compounds (Kunzemann & Herrmann, 1977; Matsumura *et al.*, 2001; Matsumura *et al.*, 2002a, 2002b; Najda *et al.*, 2008; Nakano *et al.*, 1998). A number of phyto-nutrients have been found in caraway seeds, including different vitamins, amino acids, proteins, and minerals, also starch, sugars and other carbohydrates, tannins, phytic acid and dietary fibres (Al-Bataina *et al.*, 2003). The other constituents present in this plant are fatty acids (saturated and unsaturated), triacylglycerol, polysaccharides, and lignin (Reiter *et al.*, 1998; Seidler-Lozykowska *et al.*, 2010). Carvone and limonene are usually reported as the main phytochemicals present in caraway seeds. The other important compounds extracted usually from hydro/steam distillation include: carvacrol, α-pinene, γ-terpinene, linalool, carvenone, and *p*-cymene (Raal *et al.*, 2012; Razzaghi-Abyaneh *et al.*, 2009; Simic *et al.*, 2008). Analysis of the caraway seed essential oils showed they varied in different regions and climates (Laribi *et al.*, 2013). Detected phytochemicals are mostly phenolic and aromatic compounds including monoterpenes (hydrocarbons/oxygenated), oxygenated sesquiterpenes, aldehydes, ketones, and esters (Johri, 2011).

2.3.7 Biological Activities, and Therapeutic Uses of Caraway

As discussed earlier, since ancient times, caraway and its derivatives have been widely and commonly applied as traditional medicinal plants for treating different health problems in different cultures. A variety of the therapeutic properties of caraway mentioned in traditional medicine have been investigated and confirmed experimentally. Recently, significant developments in the pharmacological assessments of caraway have been reported resulting in several recommended therapeutic activities for this plant. The main reported pharmacological actions include anti-oxidant, anti-microbial, anti-carcinogenic/anti-mutagenic, anti-diabetic/hypoglycemic, hypolipidemic/anti-hyperlipidemic, diuretic, estrogenic/anti-osteoporotic, and immuno-modulatory. Caraway is also reported to have other functional properties, including: larvicidal, anti-bacterial, and anti-fungal activities, and health promoting effects on the central nervous system with adaptogenic property (anti-stress), as well as carminative and laxative for gastrointestinal conditions, anti-dyspeptic, anti-ulcerogenic, anti-asthmatic, antitussive, and anti-spasmodic activity. Furthermore, it is used industrially, in cosmetics, and also as fumigant, molluscicide, insecticidal, or pesticide (Lim, 2013).

The diversity of bioactivities expressed by caraway preparations is attributed to the multiplicity of bioactive constituents, hence its description as having a “hot” nature in some medical systems, attributed to the high content of essential oils, flavonoids, and phenolic compounds present (Charles, 2013; Khare, 2008; Sadowska & Obidoska, 2003). However, linking a certain biological activity to a particular compound, which is the classical Western reductionist approach, has remained a challenging issue. It is more probable that multiple targeting (network pharmacology) of individual compounds and the synergy between and within specific types of phytochemicals is responsible for the

diverse notable pharmacological properties. Nonetheless, the biological actions reported for caraway compounds support the traditional medicinal properties (Johri, 2011; Khan & Abourashed, 2011).

2.3.7.1 Anti-Obesity Activity of Caraway

There are a number of evidences concerning the antiobesity potential of caraway. In an animal study, administration of carvacrol inhibited obesity in diet-induced obese mice. In this experiment, the weight gain decreased remarkably in obese mice fed with high-fat diet. Also, significant reduction was observed in their visceral fat-pad weights and plasma lipid levels. These biological changes are assumed to be linked with inhibitory effect of carvacrol on visceral adipogenesis, inhibiting formation of pro-inflammatory cytokines in visceral adipose tissues (Cho *et al.*, 2012). These findings suggest that carvacrol supplementation can induce weight loss through regulating gene expressions and modulating adipose tissue proteins involved in adipogenesis, thermogenesis and inflammation, reversing obesity.

With respect to the anti-inflammatory effect of caraway, several studies have stated inflammation as one of the major risk factors linked with obesity, also anti-inflammatory compounds have shown positive effect on weight loss (Barness *et al.*, 2007; Bastard *et al.*, 2006; Clement *et al.*, 2004; Dandona *et al.*, 2004; Dandona *et al.*, 2005; Xu *et al.*, 2003; Yang *et al.*, 2001). Besides, different studies have reported anti-inflammatory potential of caraway derivatives. A recent research has reported lipopolysaccharide-induced inflammation in macrophage cells. In this study, administration of limonene showed suppressing effect on formation of pro-inflammatory cytokines and inflammatory mediators (Yoon *et al.*, 2010). Other studies have reported anti-inflammatory effect of

caraway ingredients (Can Baser, 2008; Johri, 2011) such as carvone and limonene on skin through treating inflamed eczema (Sadowska & Obidoska, 2003), also in gut due to inhibiting several M.O that could cause inflammation related to digestive system such as gastritis (Simic *et al.*, 2008). Moreover, hypoglycemic and hypolipidemic property of caraway reported in several studies is another prove for anti-obesity potential of caraway as these biological activities are inter-correlated with obesity and weigh loss (Lebovitz, 2003). In addition, a recent patent has mentioned caraway as one of the potent plants for prevention or treatment of obesity due to containing camphene as a bioactive ingredient (Park, 2013). In another patented formula, caraway seeds are recommended as one of the ingredients in the rye bread with non-absorbable lipid binding activity (Furda, 1980).

2.3.8 Safety and Toxicity of Caraway

The safety of a traditional medicine is frequently assumed based on historical use, and rarely scientifically established through a well-constructed clinical trial. This is especially an issue when different preparations are made which do not follow traditional methods, an aqueous extract, rather than an expressed, or steam-distilled, essential oil for example. Possible therapy constraints (dose, route of administration, etc.) must therefore be deliberated as limiting aspects based on clinical evidence. Presenting products of this plant as a safe and harmless substitute therefore involves examination of the probable side-effects of consuming caraway. Research shows that a combination of caraway seed oil and peppermint oil (50 and 90 mg, respectively) has shown skin allergy, burning sensation with eructation, and nausea in sensitive patients with functional dyspepsia (May *et al.*, 2000). In addition, due to the blood glucose-lowering activity of caraway, diabetics and patients taking drugs, plant medicines, or supplements with hypoglycemic property should consume this plant with caution. Such negative reports are rare, and there is no

sufficient evidence regarding the negative reactions of caraway essential oil in humans (Nicotra, 2012).

On the other hand, several studies have described the safety of caraway. In an animal study, the effects of different doses of caraway seed powder (30, 60, and 90 mg/kg body weight) on the formation of aberrant crypt foci in DMH-induced colon cancer in rats were examined, with the result that no clinical signs of toxicity were detected in the treated rats (Kamaleeswari *et al.*, 2006). The potential hepatoprotective ability of caraway oil extract was assessed through the carbon-tetrachloride-induced hepatotoxicity test in mice. The findings showed that this plant extract probably exhibits a hepatoprotective effect through maintaining the activity of xenobiotic detoxifying enzymes, including glutathione *S*-transferase (GST), and glutathione peroxidase (GSH-Px), lowering GSH, and inhibiting lipid peroxidation (Naderi-Kalali *et al.*, 2005; Samojlik *et al.*, 2010).

Another animal study reported the reno-protective potential of caraway showing protective activity of this plant against kidney toxicity. In this experiment, administration of CWE in high dosage (60 mg/kg) in rats, exhibited nephron-protection against STZ-induced diabetic nephropathy (Sadiq *et al.*, 2010). Also, findings showed reduction in the raised glucose, serum urea, creatinine, total urinary volume, protein micro-albuminuric, and lipid peroxidation after CWE intake. In addition, usages of CEO in septic rats induced reduction in raised kidney lipid peroxidation and plasma urea/creatinine ratio levels (Dadkhah & Fatemi, 2011). These records indicate that caraway extracts could possibly provide a protective effect in kidney and liver tissues. While these findings support the safety and tolerability of caraway extract, additional safety studies are necessary to define the suitable dosage, guidelines, cautions, and other recommendations for the usage of various caraway preparations.

In summary, concerns regarding efficacy and safety of traditional medicinal plants have become progressively significant. Hence, administration and regulation of the modern techniques and procedures is necessary for commercial and traditional applications. Accordingly, more research studies are required to understand this form of medication and guarantee its quality, safety and efficacy in clinical practice. Since there are limited studies on the antiobesity property of caraway, this study will examine the efficacy and safety of caraway water extract on overweight and obese women through a randomized triple blind placebo controlled clinical trial. The materials and methods applied for evaluating the effect of CWE, also the procedures for extraction and preparation of herbal extract and placebo, will be explained in details in the next chapter. The overall framework of this study is shown in **Figure 2.7**.

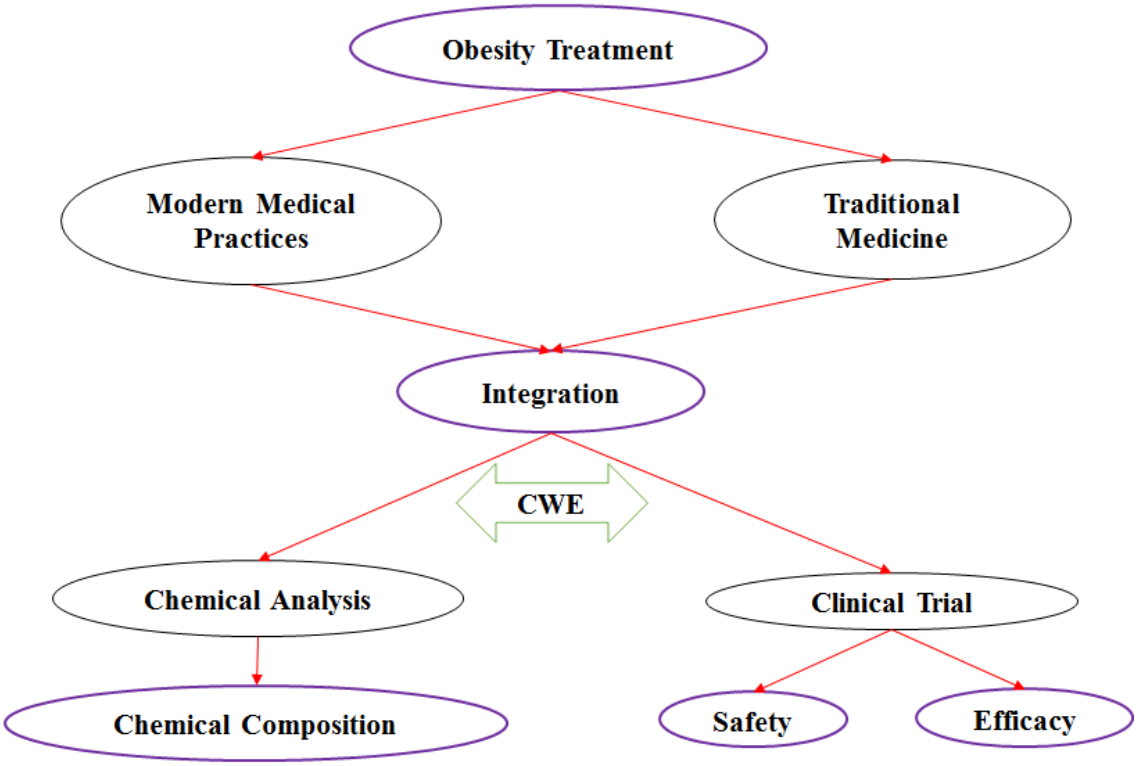


Figure 2.7: Framework of this study

CHAPTER 3: MATERIALS AND METHODS

3.1 An Overview

This chapter provides detailed explanation about the methods that had been applied by researcher to conduct this study. In accordance with the objectives of this study mentioned previously, two major experimental studies were conducted in this research. Firstly, a randomized clinical trial (RCT) was conducted to gain an in depth insight into the safety and efficacy of CWE. In addition, chemical analysis was conducted to assess the CWE compounds using GC-MS technique. **Figure 3.1** shows the flow chart of the study.

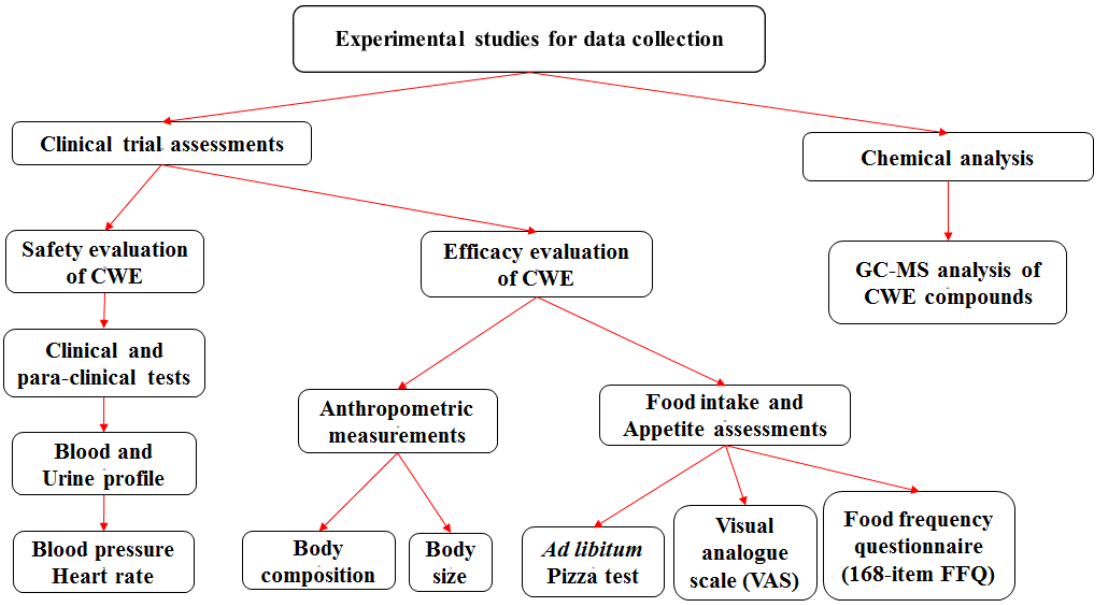


Figure 3.1: Flow chart of the measurements

3.2 Study Design

A randomized triple-blind placebo-controlled, clinical trial was designed to examine the efficacy and safety of caraway water extract (CWE) on overweight and obese women. The study was carried out at the fitness centre in Yazd, Iran, on subjects who were

recruited for aerobic trainings. Following advertisement and recruitment, the candidates were screened through health examinations by the physician and the researcher. To evaluate the effects of caraway intake, participants were randomized into test and control groups, and they were asked to consume either the prepared caraway product or the placebo preparation daily for twelve weeks. The baseline data were assessed in both groups before starting the intervention program which were finally compared with the collected data at the end of intervention, after twelve weeks. **Figure 3.2** shows the study design of the clinical trial.

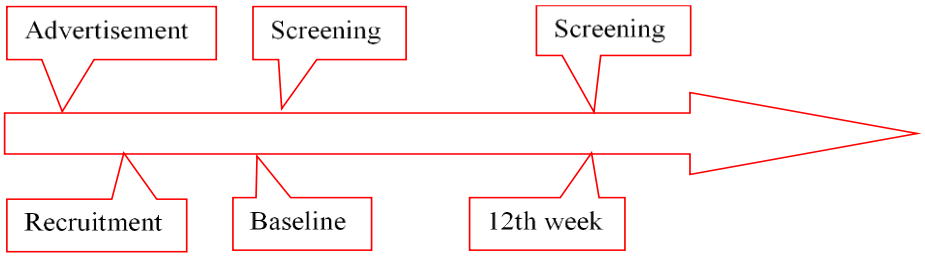


Figure 3.2: Diagram showing study design of the clinical trial

This interventional study was approved by the medical ethics committee of UMMC (University of Malaya Medical Center) under the reference number 925/15 (Appendix H). The clinical trial was conformed to the Declaration of Helsinki/Malaysian Good Clinical Practice (GCP) Guidelines. The Patient Information Sheets were provided to the participants and the written informed consent forms were signed and taken from all the volunteers. The protocol is registered at clinical trial.gov protocol registration system with the Protocol ID: NCT01833377.

3.2.1 Rationale for Study Design

To asses a causal relation, the control group with characteristics similar to test group was assigned to compare the effects of active herbal compound with the effects of placebo under similar controlled conditions. Moreover, the adequate period of time (12 weeks) which is the average duration of similar previous clinical trials (Hasani-Ranjbar *et al.*, 2013; Opala *et al.*, 2006; Vermaak *et al.*, 2011; Yun, 2010) and also extra subjects were assumed to assess the more valuable results. This is the strength of this study comparing to similar previous clinical trials.

The triple blinding (investigator, subject, and statistician) in this study could be as an outstanding parameter which decreases information and expectation bias. The investigators, subjects, and the data collectors were masked to the treatment regimens. The placebo and CWE samples were similar in appearance and the bottled samples were coded by the co-investigator who was not involved in the study. A statistician, who was not directly involved in the establishment of the groupings and the design of the trial, was provided with the codes and the data for analysis (triple-blind). This method is in line with several previous studies which have conducted triple-blind controlled trial (Hopkins *et al.*, 2004; Schulz & Grimes, 2002; Weatherley-Jones *et al.*, 2004; Wheatley, 2004).

3.3 Study Population and Subject Sampling

Overall, 110 overweight and obese women aged 20-55 years were enrolled for this study. Among the recruited candidates, only healthy women with body mass index (BMI) between 25.0 and 39.9 kg/m^2 were included and screened for the dietary intervention program. All subjects were doing moderate aerobics training for 180 minutes/week, with

an estimated energy expenditure of 1000–1200 kcal/week at different fitness centres in the city of Yazd, located in the centre of Iran.

Pregnant and lactating women, and individuals who suffered from specific health problems, were specifically excluded from this intervention. Also excluded were individuals with hypo/hyperthyroidism, a significant history or current presence of type I or II diabetes mellitus, or who were hypertensive (systolic blood pressure 140 and/or diastolic blood pressure 90), had clinically significant endocrine, hepatic, renal, or cardiovascular disease, such as impaired liver function, chronic renal disease, primary dyslipidemia, myopathy, or patients presently using drugs affecting metabolism or appetite. Individuals who have maintained a weight loss of 41 kg in the preceding three months, have the habit of not eating meals at regular intervals, have participated in another investigational study within the past 30 days, have a history of alcohol or drug abuse within the past year, have a history of sleep disorders, clinical depression, or other psychiatric or psychological conditions, and who are abnormally obese were also excluded. In general, individuals presenting with any medical condition or the use of any medication that could interfere with the conduct of the study, or which placed the prospective subject at risk, or who had a known allergy or sensitivity to any of the 'active' or 'placebo' product ingredients, were excluded. In order to reduce the possibility of allergic reactions due to the consumption of caraway, only eligible females who were familiar with caraway were selected for the intervention treatment.

3.3.1 Rationale for the Study Population

This study was carried out in Iran, as an example of an emerging country with a high outbreak of obesity and overweight (Esteghamati *et al.*, 2010; Mirzazadeh *et al.*, 2009;

Rashidi *et al.*, 2005). Importantly, the people in Iran are familiar with the use of caraway seeds or its derivatives, as a flavoring agent for culinary purposes, and also of its remedial benefits, especially for decreasing weight in the form of water extract. Furthermore, caraway products are affordable and easily available in Iran, especially at the Yazd market. In addition, caraway water extract is consumed regularly in Iran, especially in Yazd, for losing weight. Overweight and obese women were selected as the study population as the rate of obesity is mostly higher in females especially among adults. Also, women are usually more interested to attend weight loss programs than men (Sassi, 2009; Yach *et al.*, 2006).

3.4 Screening and Data Collection

For gathering the required data, three methods were used: questionnaire, face-to-face interview, and a physical examination of the prospective study subjects. All the data were collected at baseline (before starting the intervention) and after week twelve of the intervention. The possible changes in body composition, anthropometric indices, appetite, blood and urine profile, and vital parameters were investigated before and after intervention. Moreover, food intake status and physical activity level were measured during this period to ensure the harmony between the test and control groups.

For collection of data, participants were asked to fill the validated questionnaires including Food Frequency Questionnaire (168-item FFQ), International Physical Activity Questionnaire (IPAQ), and general information questionnaire (Appendix F). Food and nutrient intakes were calculated using Food Frequency Questionnaire 168-items. Adjustments of subjects to the study protocol was followed up by direct interview, observation, phone call, continuous visits to the fitness centre and returning back the

empty bottles. Primary data including medical history, nutritional and health status of the volunteers were obtained through general information questionnaire, interview and physical examination prior to starting the program. At randomization and each subsequent visit, brief physical assessments and interview were repeated for all participants.

Before starting the randomization, candidates were invited to participate in a nutritional counselling section and they were given guidelines regarding the procedures of this intervention and the benefits of participation in this study. Besides, subjects were guided how to fill the questionnaires. However, no additional advice was provided regarding the modification of dietary or exercise behaviours during the study. Volunteers were instructed to maintain their regular diet and physical activity throughout the intervention.

3.5 Study Groups and Randomization

The selected subjects were randomized, and allotted equally into CWE and placebo groups. The randomization was done through the online randomization program (www.randomization.com) which is based on the randomly permuted blocks method (Fleiss, 1986; McLeod, 1985; Wichmann & Hill, 1982). By using the first and original generator which randomizes each subject to a single treatment, all the 70 allotted subjects were randomized into blocks of 35-35 named Test and Control (Appendix C):

- Group 1 (the control group; n = 35) were given placebo samples 30 ml per day
- Group 2 (the test group; n = 35): were given caraway water extract 30 ml per day

Both the placebo and caraway extract samples were coded and were provided to the participants weekly. Also, participants were provided with measured bottles (Figure 3.3)

and were asked to dissolve 30 mL of the placebo or caraway extract with 30 mL of water. Subjects were instructed to consume caraway extract or placebo samples 20 minutes before meal (lunch) for 12 weeks intervention. They were also recommended to refrain from consuming caraway from sources other than the study products.

Figure 3.3: Measured bottles provided to participants

3.6 Sample Size Calculation

Based on similar previous studies, the required sample size was calculated using the Greenberg sample size formula (Greenberg *et al.*, 2001) with 99% level of confidence, 1% precision (alpha < 0.01) with a power level of (1-β) 0.9. The primary variable was weight and the sample size was based on a two-tailed *t* test. The standard deviation of weight in the study population was anticipated to be 14 kg which is in line with the previous studies (Hauptman *et al.*, 2000; Hollander, 2003; Muls *et al.*, 2001; Wadden *et al.*, 2012).

Greenberg (2001) formula: $$2N = \frac{4\left(Z_{\alpha} + Z_{\beta}\right)^2 \sigma^2}{\delta^2}$$

(1-β)= power (90%)

α= significance level = 0.01

Zβ=1.282 (Values of Z for 90% power)

Zα=2.58

Accordingly, a total sample (2N) of 60 subjects (30 subjects in each group) was required to detect a significant difference in weight loss of participants from baseline compared to control group. To ensure that this sample size would be available for analysis, and assuming dropouts and loss to follow up during 12 weeks intervention, 10 extra patients were randomized and included. Hence, a total of 70 overweight and obese women with BMI>25 were recruited for this study. All the results are expressed as mean ± SD (**Figure 3.4**).

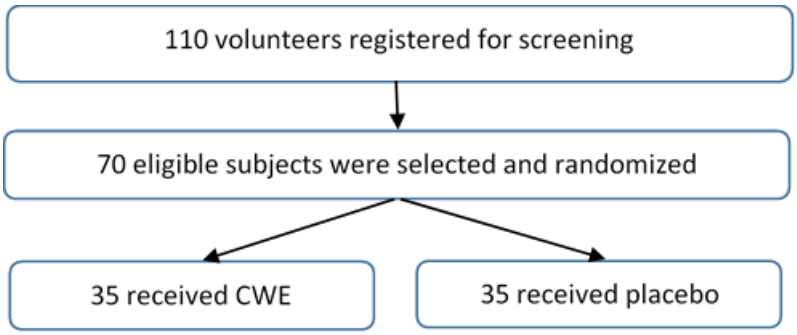


Figure 3.4: Flow chart of study groups and randomization

3.7 Demographic and Baseline Assessments

The demographic characteristics of the selected volunteers were assessed at baseline before starting the intervention. The features measured were included age (year), body weight (Kg), body mass index (BMI: Kg/m^2), physical activity level (PAL), bone mass (Kg), sleep (hours/day), basic metabolic rate (BMR), active metabolic rate (AMR), resting energy expenditure (REE), and total daily energy expenditure (TDEE).

3.7.1 Determination of Physical Activity Level

Physical activity level was estimated using the International Physical Activity Questionnaire (IPAQ) (Appendix F). The reliability and validity of this questionnaire is

reported in previous studies (Craig *et al.*, 2003; Fogelholm *et al.*, 2006; Hagströmer *et al.*, 2006). Volunteers were asked to calculate the average time (hours and minutes) spent daily on any types of physical activity including sedentary, low, moderate or high intensity. The data were presented as metabolic equivalent (MET) hours per day (kcal/kg/day) using the formula as follows (Giovannucci *et al.*, 1995):

MET value is defined as calorie need per kg body weight per hour activity divided by the calorie need per kg body weight per hour activity at rest and was calculated based on the following formula:

MET = sum of the average hours per day for each activity × MET value of each activity.

3.7.2 Determination of Basic and Active Metabolic Rate

The basic metabolic rate (BMR) (calorie per meteres^2 per hour) was calculated based on the following formula:

$$BMR = 447+ (9.25\times weight) + (3.1\times height) - (4.3\times age)]$$

Active metabolic rate (AMR) was assessed using the BIA. The BIA has divided physical activity level of the subjects into 5 categories. As all subjects had similar physical activity level (all of them practiced aerobic exercises 3days/week) during the study, the researcher had appointed all the volunteers in the moderate level.

Resting energy expenditure (REE) was calculated using the formula as follows:

$$REE= [655+ (9.6\times weight) + (1.8\times height) - (4.7\times age)]$$

The total daily energy expenditure (TDEE) was calculated by the following formula:

TDEE = (BMR×1.55)

3.8 Clinical Trial Assessments

Clinical trial assessments in this study are comprised of efficacy and safety evaluation of CWE. The efficacy studies include anthropometric measurements which consists of body size and body composition assessments. The details of materials and methods applied for assessing these parameters are described in the next section.

3.8.1 Efficacy Evaluation of CWE

3.8.1.1 Assessment of Anthropometric Indices

The anthropometric indices including height, waist circumference, hip circumference, mid-upper arm circumference (MUAC) and thigh circumference (THC) were measured in centimetres (cm) and were assessed using circular ergonomic circumference measuring tape with automatic roll-up (Seca 201). Measurements were performed early in the morning with empty stomach while the subjects were not in the menstrual cycle.

(a) ***Body Height***

Height was measured to the nearest 0.1 cm (within 0.1 cm), using Seca measuring tape. Subjects were instructed to stand barefoot with straightened legs, their heels almost together touching measurement board and looking straight ahead with Frankfurt plane horizontal and the shoulders parallel to the floor (Gibson, 2005) (**Figure 3.5**).

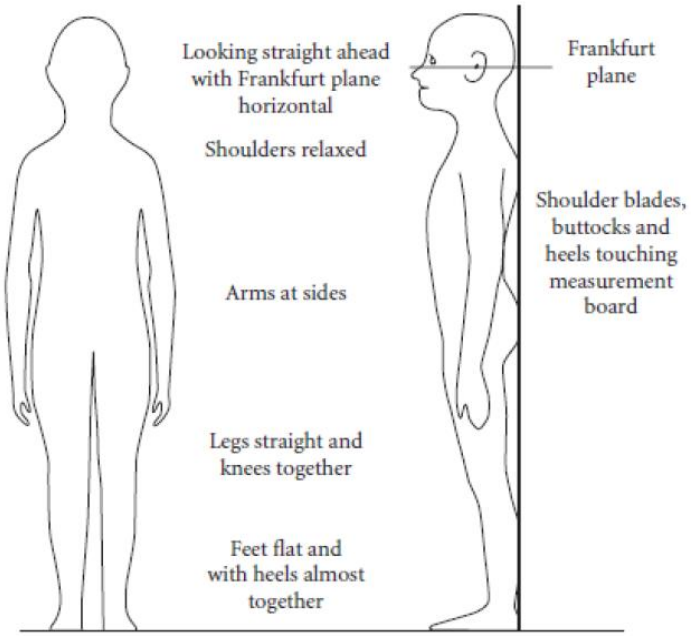


Figure 3.5: Positioning of subjects for measuring height
Adopted from: Gibson (2005)

*(b) **Waist, Hip and Mid-Upper Arm Circumference***

To assess waist, hip and mid-upper arm circumference, subjects were asked to stand with their heels together and both arms up to the sides. The waist circumference was measured by placing the measuring tape at the umbilicus point (the site between the lowest rib and the iliac crest) (National Institutes of Health, 2000; Rudolf *et al.*, 2007). **Figure 3.6** shows the measuring-tape position for waist circumference.

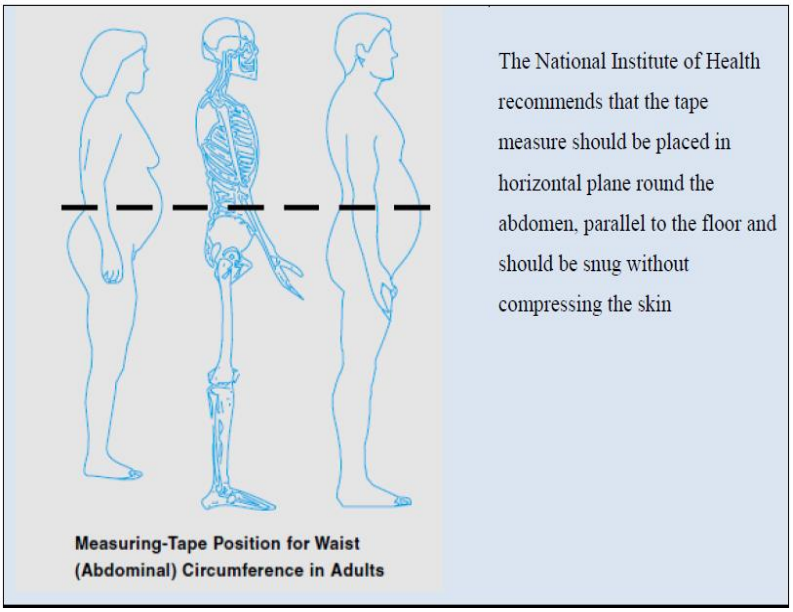


Figure 3.6: Assessing waist circumference
Adopted from: Almoosawi *et al.* (2010)

Hip circumference (HC) was measured at the maximum circumference over the buttocks (WHO, 2008). Waist to hip ratio (WHR) was then calculated using the waist and hip circumference data according the following equation:

WHR= waist circumference (cm) / Hip circumference (cm)

Mid-upper arm circumference (MUAC) was measured at the mid-point of the left arm. Subjects were asked to keep their hands up in the straight position parallel to the floor and avoid bending their arms. Also, thigh circumference was measured at the midway between the hip and the knee. Subjects were asked to open their legs to shoulder width apart.

(c) Assessment of Body Weight and Body Composition

Body weight was measured within 0.1kg intervals. Participants were weighed in light clothing and without shoes using a bioelectrical impedance analysis (BIA) (Beurer digital diagnostic scale with remote control, Model BG63, Beurer Products, Ulm, Germany). Beurer BIA has been examined for the body composition analysis in the previous studies (Das & Roy, 2010; Knechtle *et al.*, 2011; Mittal *et al.*, 2011; Nayebi, 2011; Nikooyeh *et al.*, 2011; Salekzamani *et al.*, 2011). The precision of the scale was checked regularly to ensure the scale calibration. Other body composition parameters including body fat percentage, water percentage, muscle percentage, bone density as well as AMR (active metabolic rate) were measured simultaneously. Body fat, body water and muscle mass were displayed with 0.1% graduation; bone mass and body weight with 100 gram graduation and the active metabolic rate (AMR) was measured in calorie with five activity levels. Weight measurements were performed early in the morning with empty stomach. According to the instruction of the digital scale, subjects were asked to moisten their feet by putting their feet on a damp cloth before stepping on the scale.

(d) Body Mass Index Measurement

From the height and weight measurements, BMIs were calculated according to the following formula:

$$\text{BMI} = \text{weight (kg)} / \text{height}^2\ (\text{m}^2)$$

To reduce intra-individual errors, weight and height were measured twice by the co-investigators and the mean value was used as the final data for the analysis.

3.8.1.2 Food Intake and Appetite Assessments

(a) ***Assessing Food and Dietary Intake***

Dietary calorie and nutrient intakes were assessed using the Food Frequency Questionnaire (168-item FFQ) (Appendix F). "The 168-item FFQ was a Willett format questionnaire modified based on Iranian food items. It included a list of foods (with standard serving sizes) commonly consumed by Iranians." The reliability and validity of this questionnaire has been reported in previous studies (Asghari *et al.*, 2012; Mirmiran *et al.*, 2010).

(b) ***Appetite and ad Libitum Pizza Tests***

To examine the motivation to eat and appetite rate in the participants the satiety experiment was done one week before starting the study and one week after intervention program. To study the suppressing effect of caraway water extract on appetite, volunteers were asked to record their subjective feeling of hunger, satiety, and desire to eat on a 10 cm visual analogue scales (VAS) (Jiménez-Cruz *et al.*, 2006) (**Table 3.1**). The scales were rated 1 to 10 and the end points were signed as "extremely hungry" and "extremely full". All participants were asked to rate their appetite every waking hour for three days before starting the intervention and three days after completing the intervention.

To confirm the satiating effect of caraway water extract participants from both groups were asked to go through an *ad libitum* pizza test at the end of the intervention at week 12. Subjects were instructed to have a standard breakfast at 8 am and consume 30 ml preloads of either caraway water extract or placebo 20 minutes before lunch. Volunteers were asked to have an *ad libitum* pizza meal at 1 pm as lunch and they were instructed to eat pizza slices until complete fullness and then record the number of slices they had

eaten. The amount of food intake (number of pizza slices consumed) was compared between test and control group which were further analysed by SPSS to identify the significant difference between groups.

Table 3.1: Visual analogue scale (VAS) for rating fullness and hunger

Appetite scale code*									
1	2	3	4	5	6	7	8	9	10
Extremely hungry									Extremely full

*Appetite code	Feeling of fullness/hunger
1	Extremely hungry, famished, starving
2	Feel hungry, low energy, weak
3	Want to eat now, stomach growls and feels empty
4	Hungry but could wait to eat, starting to feel empty but not there yet
5	Not hungry, not full
6	Feeling satisfied, stomach feels full and comfortable
7	Feeling full, definitely don't need more food
8	Uncomfortably full
9	Stuffed, very uncomfortable
10	Extremely full, bursting, painfully full

3.8.2 Safety Evaluation of CWE

The safety evaluation of CWE includes the clinical (blood and urine tests) and para-clinical assessments (blood pressure and heart rate). At the baseline randomization visit and after 12th week consumption of caraway water extract or placebo, candidates were asked to refer to the allotted laboratory in hospital for blood and urine test sampling for biochemical analyses. Blood sampling and analysis were taken between 07:30 and 09:30 am by an experienced technician at the Yazd Central/Reference Medical Laboratory, Yazd, Iran.

All the blood tests were conducted at the reference laboratory of Shahid Sadoughi hospital in Yazd, Iran in the fasting condition. Blood sampling were done by a trained nurse and in each sampling, 10 ml blood were taken from the forearm vein of the volunteers. The biochemical parameters were analysed using the ELITECH diagnostics Kits (SEPPIM S.A.A.-Zone Industrielle– 61500 SEES FRANCE) with the detection precision of 0.5 mg/dL within run. The procedure employed was as described in "Clinical guide to laboratory tests" by Tietz (1995). The performance data including analytical range, detection limit, sensitivity, precision and correlation were obtained at 37 °C on Selectra. The detection limit was determined according to SFBC protocol (Vassault *et al.*, 1986). For separation of serum from plasma, Sigma centrifuge (SIGMA Laborzentrifugen GmbH, Germany) were used at 3500-4000 RPM for 12 minutes at 4°C.

To assess the probable toxicity or side-effects effects of caraway water extract, the blood tests including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total (Bilirubin, T), and bilirubin direct (Bilirubin, D) were done. These blood parameters were known as the biomarkers of liver cell injury. The biomarkers of lipid metabolism were also determined which include: total cholesterol (T-C), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG). The hematological parameters (Peripheral blood/Complete blood cell count- CBC test) include: white blood cell (WBC), red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), mean corpuscular volume (MCV), Red cell distribution width (RDW-CV), Platelets count (PLT), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), and platelet distribution width (PDW). The other biochemical tests including fasting blood sugar (FBS), urea, creatinine, and uric acid (UA) as well as urine-specific gravity (USG) were analysed.

In the following section, the clinical significance, and methods used for assessing each biochemical parameter is mentioned. The other information including principle of the method, reagents composition, reference values, procedure, and calculation of each parameter is described in details at the Appendix D.

3.8.2.1 Serum Glucose Assessment

Glucose is the main source of energy for the human body. Glucose is converted either into glycogen to be stocked in the liver or into triglycerides to be stocked in fatty tissues. Glucose concentration in blood is regulated by several hormones, including two antagonists: insulin and glucagon. Quantification of glucose in blood is used to diagnose metabolic carbohydrates disorders such as diabetes, neonatal glycaemia, idiopathic hypoglycemia and pancreatic disease. The man physiological troubles were linked to hyperglycemia (type I and type II diabetes mellitus). Type I diabetes mellitus is insulin dependent and appears mainly before 30 years old. Type II diabetes mellitus is non-insulin-dependent and usually appears after 40 years old, but can occur earlier for obese people. Other diabetes have secondary origin and appear after endocrinal or hepatic diseases (Sacks *et al.*, 2002). Fasting blood sugar was determined using kits GPSL–0490/0500/0700/0507/0707 by enzymatic-colorimetric (Trinder – Kinetic) technique.

3.8.2.2 Lipid Profile

Lipid profile including determination of total serum cholesterol, serum cholesterol LDL, serum cholesterol HDL-C and triglycerides were assessed before and after twelve weeks intervention. The procedures are explained in details as the following.

(a) ***Determination of Total Serum Cholesterol***

Cholesterol is both coming from food and synthesized by the human body, mainly in hepatic and intestinal cells. Cholesterol is a component of cells and organoids membrane. It is a metabolic precursor of bile acids, vitamin D and steroid hormones. Cholesterol, insoluble molecule, circulates associated with lipoproteins (HLD, LDL and VLDL). Quantification of total cholesterol allows the detection of hypercholesterolemia, isolated or associated with hypertriglyceridemia. High cholesterol concentrations were associated with a high risk for vascular accident and apparition of atherosclerosis. The LDL/HDL ratio should be taken in consideration for evaluating the risk of developing cardiovascular diseases (Naito, 2003; Rifai *et al.*, 1999). Cholesterol was determined using kits CHSL – 0490/0500/0700 by enzymatic-colorimetric (Trinder-End point) method. The samples were serum and heparin or EDTA plasma from fasting patients.

(b) ***Determination of Serum Cholesterol LDL***

Low density lipoprotein (LDL) come from very low density lipoprotein (VLDL) hydrolysis by different lipolytic enzymes. LDL, which transports approximately 60% of total plasmatic cholesterol, is mainly taken up through specific receptors by extra-hepatic and hepatic tissues. A positive association exists between the incidence of coronary heart disease and LDL cholesterol. LDL is an atherogen lipoprotein: LDL cholesterol increase is a major cause of apparition and evolution of atherosclerosis, in particular, coronary atherosclerosis. Therefore, the treatment of elevated LDL cholesterol is the primary target of cholesterol-lowering therapy. An increase in LDL cholesterol may be seen in different pathological states including hyperlippoproteinemia types IIa and IIb, premature coronary heart diseases, hyperlippoproteinemia due to hepatic or renal disorder, to hypothyroidism and diabetes (Naito, 2003; Rifai *et al.*, 1999). Cholesterol LDL was determined using kits

LDLL – 0380 using enzymatic- selected detergent End point method. The samples were serum and heparin or EDTA plasma from fasting patients.

(c) Determination of Serum Cholesterol HDL-C

The high density lipoprotein cholesterol (HDL-C) test is an in vitro assay for the quantitative determination of HDL-C in serum. Lipoprotein is classified into LDL, VLDL and chylomicrons (CM) according to specific gravity. In recent years, in addition to total cholesterol, HDL-C has become an important tool used to assess an individual risk of developing coronary heart disease (CHD) since a strong negative relationship between HDL-C concentration and CHD was reported (Graham *et al.,* 2007; Rifai *et al.,* 1999). HDL-C has been assayed by ultracentrifugation, gel filtration, electrophoresis, high performance liquid chromatography (HPLC), precipitation, and direct process (Gordon *et al.*, 1977). Recently, direct process has been widely used.

Here, stable liquid Reagent-Immunoinhibition method has been used to determine Cholesterol HDL using kits HDLL – 0507 D8. HDL-C is a liquid type reagent that assays HDL cholesterol in serum directly by employing antibody.

(d) Determination of Triglycerides

Triglycerides constitute 95% of tissue storage fat and their main role is to provide energy for the cell. They were synthesized both in the intestine from dietary fats and in liver from dietary carbohydrates, and were then transported in blood by chylomicrons and VLDL. High serum triglyceride levels were associated with important risks of atherosclerosis. They can be due to several diseases like different lipid metabolism disorders (hyperlippoproteinemia, lipase activity deficiency), also to diabetes, renal or

endocrine disorders (Naito, 2003). Triglyceride was determined using kits TGML – 0425/0515/0700 by enzymatic-colorimetric (End point) method.

3.8.2.3 Hematological Analysis (CBC)

The samples were used for complete blood cell count (CBC) by automated hematology analyzer (Sysmex blood cell counter auto-analyzer model KX21; Japan). The analysis parameters include WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT (platelet), RDW-CV, PDW, and MPV (Appendix D).

Concerning the side effects of CWE intake, changes in a number of blood parameters including liver functions (ALT, AST, ALP, and bilirubin) as well as creatinine and urine-specific gravity along with changes in SBP, DBP, and HR were examined. Furthermore, participants showing any probable allergy or clinical side-effects related to consumption of CWE or placebo would be excluded from continuing the intervention. The details of these examinations will be discussed in the next section.

3.8.2.4 Liver Function Tests

(a) ***Determination of serum alanine aminotransferase (ALT/GPT)***

Alanine aminotransferase (ALT) also known as glutamate pyruvate transaminase (GPT) is a transaminase. ALT catalyses the transfer of the amino group of L- alanine to α-ketoglutarate to give L-glutamate. The highest levels were found in liver and the kidneys, and in smaller amounts in heart and skeletal muscle. ALT concentration is increased when hepatic cells were damaged (liver cell necrosis or injury of any cause). Indeed, viral and toxic hepatitis induced a marked elevation of ALT activity in serum. Intake of alcohol, delirium tremens, and administration of various drug induce slight or

moderate elevation of ALT. ALT concentration in serum is also slightly increased in various conditions such as muscular dystrophy, haemolytic disease and myocardial infarction. ALT is more liver specific than aspartate aminotransferase (AST). Measurement of both AST and ALT has same value in distinguishing hepatitis from other parenchymal lesions. ALT serum level can decrease in case of vitamin B6 deficiency (Tietz, 1995). Alanine aminotransferase was determined using kits ALSL – 0410/0430/0510 by IFCC method (Kinetic-UV) without pyridoxal phosphate (P-5'-P).

(b) ***Determination of Serum Aspartate Aminotransferase (AST/GOT)***

Aspartate aminotransferase (AST) also known as glutamate oxaloacetate transaminase (GOT) is a transaminase. AST catalyses the transfer of the amino group of L- aspartate to α-ketoglutarate to give L-glutamate. AST is widely distributed in the body, but the highest levels were found in heart, liver, skeletal muscles and the kidneys. Damages to cells of these tissues induce AST increase in serum. In case of fulminant forms of hepatitis, especially viral hepatitis the enzyme level is markedly elevated. In case of myocardial infarction, AST activity increases and reaches a peak after 18-24 hours. The activity falls back to normal after four to five days, provided no new infarct has occurred. The following pathological states were examples of disorders also resulting in an increase of enzyme activity: liver cell necrosis or injury of any cause (for example, intake of alcohol, delirium tremens, and administration of various drug induce moderate AST elevation), alcoholic hepatitis, muscular dystrophy and gangrene, infectious mononucleosis, acute pancreatitis, heart affection as myocarditis or pericarditis and pulmonary emboli. On the contrary, AST serum level can decrease in case of vitamin B6 deficiency (Tietz, 1995). Aspartate aminotransferase was determined using kits ASSL –

0410/0430/0510 by IFCC method (Kinetic-UV) without pyridoxal phosphate (P-5'-P). The samples were serum free from haemolysis or heparinized plasma.

(c) Determination of Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) corresponds to a group of phosphatases that display maximum activity at alkaline pH. ALP is widely distributed in liver, osteoblasts, intestinal epithelium, kidneys, and placenta. The rate of ALP rises physiologically for children and teenagers during periods of active growth, as well as for women during the third trimester of pregnancy. Marked increases of ALP rate were observed in cases of extra-hepatic obstruction (gallstones, tumors, etc.) and bone diseases such as Paget's disease and osteogenic bone cancer. ALP activity can also increase moderately in the event of intra-hepatic obstruction, hepatitis, cirrhosis, or along with rickets, osteomalacia, hyperparathyroidism, healing of bone fractures (Moss & Henderson, 2001). Alkaline phosphatase was determined using kits PASL – 0400/0420/0500 based on DGKC and SCE (Enzymatic-Kinetic) technique (Hørder *et al.*, 1979). The Samples were serum free from haemolysis or heparinized plasma.

(d) Determination of Bilirubin

Approximately 80-85% of the bilirubin produced is derived from the heme moiety of the hemoglobin released from aging erythrocytes in the reticuloendothelial cells. Bilirubin bound to albumin, is transported into the liver where it is rapidly conjugated with glucuronide to increase its solubility. Then it is excreted into biliary canaliculi, and hydrolysed in the gastrointestinal tract. Unconjugated bilirubin serum concentration increase in case of over production of bilirubin (acute or chronic haemolytic anemia) and in case of disorders of bilirubin metabolism and transport defects (impaired uptake by

liver cells: Gilbert's syndrome; defects in the conjugation reaction). Reduced excretion (hepatocellular damage such as hepatitis and cirrhosis) and obstruction to the flow of bile (most often produced by gallstone or by tumors) induce an important elevation of conjugated bilirubin and in a minor extent an increase of unconjugated bilirubin (conjugated hyper-bilirubinemia) (Sherwin, 2003). Bilirubin was determined using kits BITD–0600 (total & direct 4+1), BIDI-600 (DIRACT 4+1) and BITO-600 (total 4+1) by Malloy-Evelyn modified (Endpoint) method.

3.8.2.5 Kidney Function Tests

(a) ***Determination of serum urea***

Urea is the major metabolite product of protein catabolism. The biosynthesis of urea from ammonia is exclusively carried out by hepatic enzymes. More than 90% of urea is excreted through the kidneys, with the remainder excreted through the gastrointestinal tract or skin. Blood urea concentrations can be increased by numerous factors linked to pre-renal causes (increased protein catabolism, as in haemorrhage into gastrointestinal tract, shock, some chronic liver disease) or renal/postrenal causes (acute or chronic renal diseases, postrenal obstruction to urine flow). Uraemia is also increased by high protein diet, state of dehydration, muscle wasting (as in starvation). The determination of urea rate is used together with the determination of creatinine rate to discriminate-between-prerenal (normal Creatinine) and renal/postrenal (increased Creatinine) disorders (Newman & Price, 2001). Urea was determined using kits CHSL–0400/0420/0500/0407/0427/0507 by enzymatic-UV (Kinetic) method.

(b) ***Determination of Serum Creatinine***

Creatinine is the waste spontaneous product of creatine metabolism. It is an excellent marker of the renal function. The serum Creatinine rate tends to remain constant. A high serum creatinine rate (associated to a high urea rate) corresponds to a decrease in glomerular filtration rate (GFR). The serum Creatinine test is more reliable than the urea test. Indeed, the urea serum rate is affected by factors such as diet, dehydration degree and protein metabolism (the serum Creatinine rate is not influenced by these factors). The test of Creatinine clearance can also be used to measure the GFR. In the case of renal transplantation, any increase in serum Creatinine, as little as it may be, can reflect the rejection of the transplant. An increase of Creatinine serum and urine can be the sign of muscular necrosis (Newman & Price, 2001). Creatinine was determined using kits CRCO – 0600/0700 by colorimetric, Jaffe (Kinetic) method.

(c) ***Determination of Uric Acid***

Uric acid is the major product of the catabolism of endogenous and exogenous (dietary) purine nucleosides (adenosine and guanosine). This transformation mainly occurs in the liver. Approximately, 75% of uric acid is eliminated by kidneys; the remainder is secreted into the gastrointestinal tract, where it is degraded by bacterial enzymes. Uric acid is not very soluble in water; urate crystals can occur in urines when the concentration is abnormally high. It can also happen in plasma, crystals then deposit essentially in joints, which induce intense inflammatory responses (gout). Some causes for increasing uric acid rate in serum were: increasing of purines synthesis, metabolic disorders, nutritional troubles, increasing of nucleic acid turn-over in case of proliferation of tumor cells, leukaemia, psoriasis, cytotoxic drugs, renal failures, etc. The increased uric acid is a physiological biomarker of obesity, diabetes mellitus, hypertension and

cardiovascular diseases (Masuo *et al.*, 2003). Uric acid was determined using kits AUML – 0420/0500/0700 by enzymatic –colorimetric (Trinder. Endpoint) method.

3.8.2.6 Urine-Specific Gravity (USG) Assessment

The urine tests are part of screening tests for detection of diabetes, metabolic abnormalities, liver diseases, biliary and hepatic obstructions, haemolytic diseases and diseases of kidney and urinary tracts. The well sterilized and clean vessels were provided to patients to collect the urine samples. Test strips (Medi-Test Combi 11) were used for rapid determination of density in urine through reflectophotometrical evaluation with URYXXON 200. The qualified personnel use fresh and un-centrifuged urine. The instructions are as the following:

1) Shake urine sample well before use
2) Dip the test strip for approximately 1 second into the urine
3) Draw it across the rim of the container to remove excess urine
4) Place the test strips on to the instruments according to the instructions for use in the manual.

The tests pads were reflectophotometrically evaluated and the results were printed out. The results obtained with the URYXXON 200 correspond to the concentration ranges indicated on the colour chart after 30-60 seconds. Colour changes that take place after more than two minutes are of no significance. The urine should not be more than 2 hours old when tested. Due to the fact that the human eye evaluates colour changes somewhat differently than a URYXXON 200 reflectometer, there can also be differences between these two evaluations.

3.8.2.7 Para-Clinical Assessments (Blood pressure and Heart Rate)

Vital parameters including blood pressure and heart rate were measured by a trained physician using a calibrated mercury sphygmomanometer, stethoscope and an appropriate cuff sizes on the sitting subject's right arm after a 10-min rest. Systolic as well as diastolic blood pressure was defined according to phase I and phase V Korotkoff sounds, respectively.

3.8.3 Statistical Analysis

Values for each subject were standardized for each dependent variable to remove outliers using Z-scores, and the normal distribution was tested using the Kolmogorov-Smirnov test. Student's t-test, with a 99% confidence interval, was applied to identify the significant differences in values between groups, and the paired t-test was used to examine mean differences within each group during the 12-week treatment period. All statistical analyses were performed using SPSS software version 18.0.0 (SPSS Inc., Chicago, IL, USA), and all data are expressed as mean ± standard deviation (SD); p values less than 0.01 were considered to be significant and equal variances were assumed.

3.9 Preparation of Herbal Extract and Placebo

The caraway extract samples (0.1 w/v) obtained from the Baharan Company, Yazd, Iran (Industrial Ministry License no. 28/1232 and Health Ministry License no. 35/10500) were extracted from the seeds of caraway through steam distillation. The placebo was prepared by dissolving edible caraway essence (Givaudan Flavours Co., Kempthal, Switzerland) in drinking water (1% g/L) which was identical with caraway extract in

appearance and flavour. Subjects were provided with measured bottles and were asked to dissolve 30 mL of the placebo or caraway extract with 30 mL of water.

3.10 Extraction Procedure of CWE

Previously, herbal distillates were considered as a by-product of distillation while today, they are highly used as an outstanding co-product of aromatic herbs as they contain bioactive compounds (Teo *et al.*, 2010). Moreover, the steam distillation of aromatic plants is known as one of the most economical, simplest and fastest method of extraction commonly used (Douglas *et al.*, 2006).

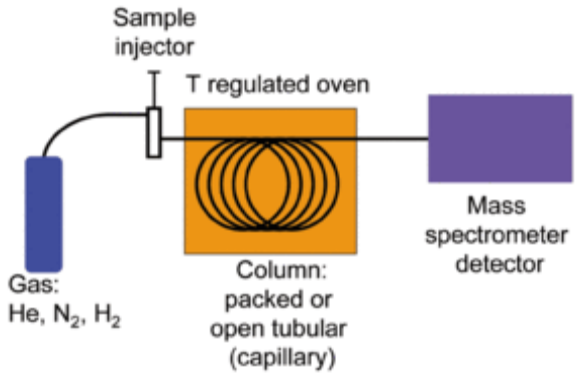
In this study, steam distillation was carried out for producing the aqueous solution or water extract of caraway which is also called as "herbal distillate, herbal water or essential water". The distillation procedure was taken around 5-6 hours in 100-120 ^{o}C at the 76 cm Hg pressure (equal to the atmosphere pressure). All the tanks were stainless steel with the 1000-2000 litres capacity which were made by the Taghtiran Company (Kashan) and Shayansazan (Yazd), Iran. The caraway seeds were collected from the plains between Khorasan and Kerman provinces including Kerman, Yazd and Shiraz cities in the south of Iran. The seeds were harvested during September and October and stored in storage rooms until processing in the Baharan manufacturing plant (Appendix E).

3.11 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The phytochemical constituents present in CWE were identified using GC-MS analysis with a flame ionization detector (FID), and extracted by HS-SPME with subsequent hexane extraction. The capillary gas chromatographic profiles of the CWE constituents were reported as their retention time compared with the MS of standard

compounds. The GC-MS analysis was performed on the Younglin gas chromatograph series YL 6100 with a capillary column DB-5MS (J&W Scientific) fused silica (60 m × 0.25 mm i.d. × 0.25 μm film thickness). The purified helium was applied as the carrier gas and the SPME Fibre, CAR/PDMS fiber (75μm), was used for volatile extraction. **Figure 3.7** shows the schematic of GC-MS instrument.

The operating conditions were included hydrogen flow 30 ml/min, air flow 300 ml/min, helium flow 0.8ml/min; and the injector and detector temperatures were 260, and 280°C, respectively. The GC oven was held at 40°C for 1 min, it was then ramped at 15°C/min to 90°C and held for 4 min, and finally ramped at 10°C/min to 170 °C/min and held for 4 min. The data related to the isolated compounds were shown on the screen of a compact PC computer connected to the GC-MS instrument and then identified from MS Libraries NIST 0.5 L (Adams, 2007).



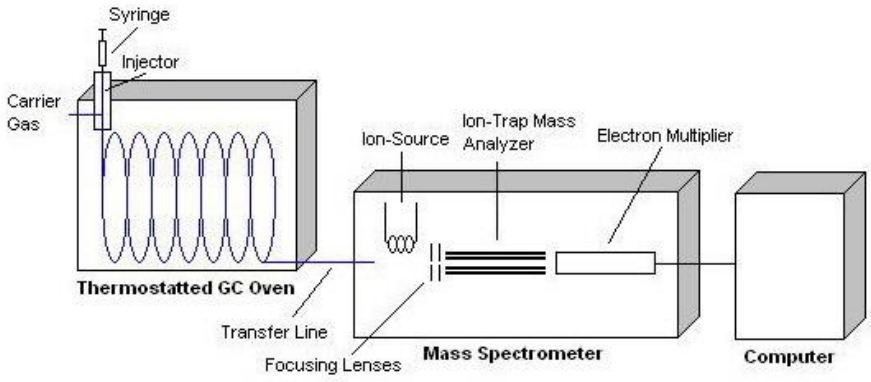


Figure 3.7: GC-MS schematic
Adopted from: (http://chemwiki.ucdavis.edu)

CHAPTER 4: RESULTS

4.1 An Overview

Of the 110 overweight and obese women who originally registered for screening, only seventy were deemed eligible to have met the study requirements and constraints. The selected subjects were randomized and assigned equally to the CWE and placebo groups. Of the selected participants, ten of the subjects- six in the placebo group and four in the CWE group- failed to complete the study. Of these ten subjects who dropped-out during the intervention (**Figure 4.1**), one was excluded due to pregnancy, three due to personal reasons, two in the placebo group because of lack of weight loss, and four subjects could not be followed-up during the study. At the termination of the study, therefore, sixty of the seventy patients completed the full twelve weeks of treatment.

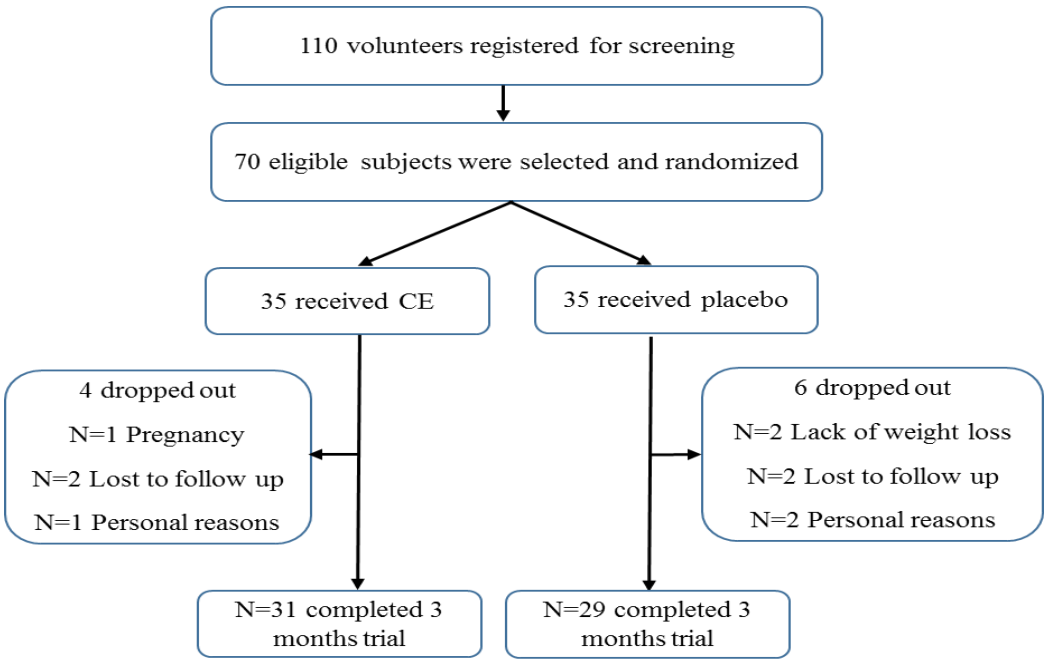


Figure 4.1: Follow-up of subjects involved in the clinical trial

4.2 Demographic and Baseline Characteristics of The Study Population

The demographic and baseline features of the participants in the study are illustrated in **Table 4.1**. Approximately 54% of the participants were overweight, and 46% were obese. All of the participants had abdominal obesity (waist circumference >88 cm). The average values (mean ± SD) of age, body weight, and body mass index (BMI) of the subjects were 37.1 ± 8.6 years, 75.4 ± 11.7 kg, and 29.8 ± 4.1 kg/m^2, respectively. The subjects had an average (mean ± SD) 44 ± 2.6 (kcal/kg/day) of physical activity level (PAL) and 7.9 ± 1.4 hours sleep during a 24-hour period.

There were no significant differences in any of the demographic variables measured including age, height, weight, BMI, and bone mass between the study and control groups. Similarly, no significant differences were observed in sleep hours, PAL, basic metabolic rate (BMR), active metabolic rate (AMR), resting energy expenditure (REE), and total daily energy expenditure (TDEE) measurements between the CWE group and the placebo group at baseline. Also, there were no significant differences in the average levels of body composition, anthropometric indices, food intake and appetite, and clinical and para-clinical assessments between the two groups at baseline. Hence, there were homogeneity and balance between the two groups at baseline.

Table 4.1: Demographics and baseline characteristics of the study population randomized to the placebo or CWE groups (n=35)

Variables	Placebo Group Mean ± SD	CWE Group Mean ± SD	p-value
Age (years)	37.0 ± 7.9	37.2 ± 9.3	0.91
Height (cm)	158.2 ± 4.9	159.7 ± 6.2	0.25
Weight (kg)	74.9 ± 11.7	76.0 ± 11.8	0.70
BMI (kg/m^2)	30.4 ± 4.7	29.2 ± 3.4	0.24
Bone Mass (kg)	7.8 ± 1.1	8.1 ± 1.1	0.35
Sleep (hours/day)	7.9 ± 1.6	7.9 ± 1.3	0.97
PAL (kcal/kg/day)	43.7 ± 2.5	44.4 ± 2.8	0.26
BMR ($kcal/m^2$/hour)	1474.4 ± 123.5	1488 ± 154.4	0.69
AMR ($kcal/m^2$/hour)	2241.4 ± 216.3	2176.6 ± 260.5	0.30
REE (kcal)	1453.2 ± 133.3	1503.1 ± 127.4	0.15
TDEE (kcal)	2236.1 ± 206.9	2308.3 ± 193.2	0.17

Abbreviations: BMI: body mass index; PAL: physical activity level; BMR: basic metabolic rate; AMR: active metabolic rate; REE: resting energy expenditure; TDEE: total daily energy expenditure

4.3 Comparison Within and Between CWE and Placebo Groups During the Trial

The changes in variables over the twelve-week intervention period within and between the CWE and the placebo groups are also calculated. The alterations in variables including body composition and anthropometric indices, as well as clinical and para-clinical assessments are discussed in details in the following section.

4.3.1 Effect of CWE on Weight and Body Composition

The changes in body composition variables over the twelve weeks of the trial for the CWE and placebo groups are summarized in **Table 4.2**. Following the twelve weeks of treatment, the mean body weight, BMI, and body fat (BF) percentage, had significantly decreased compared with placebo, whereas in the placebo group, these values increased slightly, although changes were not significant. Therefore, CWE possibly had a positive effect on inducing weight and fat loss.

Findings show that in the CWE group there was an average weight loss of 1.9 kg, while in the placebo group, an average weight gain of 0.81 kg was observed. Also, the BMI showed a significant reduction of 0.8 in the CWE group, while in the placebo group, the BMI showed an increase of 0.2 which was not significant. The BF percentage showed a significant reduction of -0.7% in the CWE group, while in the placebo group BF showed an increase of 0.2%. In contrast, body muscle (BM) percentage increased significantly in the CWE group after treatment (0.2%), while in the placebo group, no significant changes were observed. Furthermore, the difference in growing body muscle after the twelve-week intervention was considerably higher in the subjects consuming CWE, compared to the placebo group. The percentage of body water (BW) reduced in both groups (-0.01% in the CWE and -0.2%, in placebo group), and was not significant, either within, or between, groups.

4.3.2 Effect of CWE on Anthropometric Indices

The measured anthropometric indices are also presented in Table 4.2. After the twelve-week intervention, the waist circumference (WC) and the waist to hip ratio (WHR), were reduced significantly only in the CWE group, while the average levels of thigh circumference (THC) and mid-upper arm circumference (MUAC) showed significant reduction in both treatment groups. In addition, the level of reduction in all body size measurements and anthropometric indices were significant between the CWE group and the placebo group.

After the intervention period, the WC in the CWE group showed a considerable drop of -6.2 cm, while in the placebo group the reduction was not significant (-0.1 cm). Also, the WHR was reduced (-0.03 cm) in the CWE group, while no significant changes were

observed in the placebo group. The reduction in THC was significant in both groups (-5.4 cm in the CWE and -1.9 cm, in placebo group) after treatment, and the difference in reduction between the two groups was significant. Mid-upper arm circumference (MUAC) showed a significant reduction of 2.2 cm in the CWE group, while the reduction in MUAC in the placebo group was only 0.8 cm, and the decrease in MUAC during the intervention was significantly higher in CWE group compared to the placebo group. Accordingly, the CWE showed greater efficacy compared to placebo for each of the primary outcome variables with respect to the anthropometric indices and body composition measurements, except for body water.

Table 4.2: Changes (Mean ± SD) in body composition and anthropometric indices between and within groups after twelve weeks intervention

Variables	Week 0 (baseline)		Week 12	
	Placebo (n=29)	CWE group (n=31)	Placebo (n=29)	CWE group (n=31)
Body composition				
Weight (kg)	72.0 ± 10.7	76.9 ± 12.2	72.8 ± 10.8	75.0 ± 12.2*
BMI (Kg/m2)	28.3 ± 2.6	30.7 ± 4.7	28.5 ± 2.8	29.9 ± 4.7*
BF (%)	33.8 ± 2.4	35.4 ± 3.6	34.0 ± 2.5	34.7 ± 3.7*
BM (%)	31.8 ± 1.3	31.4 ± 1.6	31.8 ± 1.3	31.6 ± 1.6*
BW (%)	48.3 ± 1.9	47.2 ± 2.6	48.1 ± 1.8	47.2 ± 2.7
Anthropometric indices				
WC (cm)	91.3 ± 7.3	96.0 ± 10.2	91.2 ± 7.9	89.8 ± 8.6*
WHR	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1*
THC (cm)	59.7 ± 4.5	61.7 ± 5.8	57.9 ± 4.6 Ł	56.3 ± 5.6 Ł *
MUAC (cm)	31.0 ± 3.4	32.4 ± 3.3	30.2 ± 3.2 Ł	30.2 ± 2.7 Ł *

Abbreviations: BMI: body mass index; BF% body fat percentage; BM%: body muscle percentage; BW%: body water%; WC: waist circumference; WHR: waist to hip ratio; THC: thigh circumference; MUAC: mid-upper arm circumference

*, Significantly different from baseline compared to placebo ($p < 0.01$), independent samples t-test

Ł, Significantly different from baseline within group ($p < 0.01$), paired samples t-test

4.3.3 Effect of CWE on Food and Energy Intake

The mean (mean ± SD) weight (g) and energy (kcal) of food consumed daily, and the average daily intake of protein (g), carbohydrate (g), and total fat (g) were calculated according to the Food Frequency Questionnaire (FFQ-148 items), and the results are shown in **Table 4.3**. At baseline, there were no significant differences in any of the food

and energy intake variables measured, including weight, energy, protein, carbohydrate, and total fat between the CWE and groups, which indicates a good matching between two groups. After the twelve-week intervention, the average values of all food intake variables showed a reduction in both groups, however, the CWE group showed a greater drop in all variables compared to the placebo group. At the end of the intervention period, all of the variables, except for energy intake, showed significant reduction in the CWE group from baseline, while the drop in the placebo group was not significant. However, there was no significant difference in the reduction between the CWE group and the placebo group in any of the measured variables at week twelve, except for carbohydrate intake, which was remarkably decreased in the CWE group compared to the placebo group.

Table 4.3: Changes (Mean ± SD) in daily total energy and macro-nutrient intake between and within groups after twelve weeks intervention

Variables	Week 0 (baseline)		Week 12	
	Placebo	CWE group	Placebo	CWE group
#Weight (g)	1964 ± 499	2525 ± 1303	1938 ± 461	2109 ± 663
Energy (kcal)	2408 ± 440	2496 ± 589	2346 ± 400	2270 ± 596 Ł
Protein (g)	79 ± 15	88 ± 25	79 ± 17	80 ± 25 Ł
Carbohydrate (g)	319 ± 70	345 ± 80	313 ± 69	315 ± 82 Ł *
Total fat (g)	99 ± 29	94 ± 29	95 ± 25	85 ± 28 Ł

#Weight (g): The weight (g) of daily food items consumed by participants
*, Significantly different from baseline compared to the placebo ($p < 0.01$), independent samples t-test
Ł, Significantly different from baseline within group ($p < 0.01$), paired samples t-test

The weight (g) of daily food items consumed by participants was reduced in both groups (-416.1±917.2 g in the CWE group and -26.1±129.5 g, in the placebo), and was not significant either within or between groups. The food energy intake showed a significant reduction of 225.4±276.3 g in the CWE group, but only a decrease of 62.8±181.1 g in the placebo group which was not significant. The reduction from baseline in protein intake was also significant in the CWE group (-7.8±9.0 g), but was not significant in the placebo group (-0.5±13.2 g). Similarly, the total fat in the CWE group

showed a significant reduction from baseline (-4.5±12.8 g), while there was no significant reduction in the placebo group (-9.2±11.6 g). The carbohydrate intake in the CWE group was reduced significantly from baseline (-30.1±37.0 g), while the reduction in the placebo group (-5.9± 18.9 g) was not significant. However, a significant difference was observed between the CWE group and the placebo group in carbohydrate intake at week 12. Hence, it is plausible that CWE has a positive effect on reducing food and energy intake, especially the intake of carbohydrates, which might help in losing weight in subjects naturally.

4.3.4 Effect of CWE on Appetite

The average (mean ± SD) appetite of the participants in both groups measured at baseline and after twelve weeks of treatment, and the average number of pizza slices consumed by the subjects (*ad libitum* pizza test) at the end of the intervention period were calculated, and the results are shown in **Table 4.4**.

Table 4.4: Changes (Mean ± SD) in appetite measurements between and within groups after twelve weeks intervention

Variables	Week 0 (baseline)		Week 12	
	Placebo Group	CWE group	Placebo Group	CWE group
Pizza test Pizza slices no.	-		4.7 ± 1.0	3.9 ± 1.1*
VAS test Appetite	4.0 ± 1.1	4.3 ± 0.9	4.0 ± 1.1	3.3 ± 1.0 Ł *

*, Significantly different from baseline compared to the placebo ($p < 0.01$), independent samples t-test
Ł, Significantly different from baseline within group ($p < 0.01$), paired samples t-test

The average appetite was decreased significantly only in CWE group during the twelve weeks of study. In the CWE group, significant appetite loss of 1.0 was observed, while the reduction in the placebo group was not significant (-0.01). Correspondingly, the appetite loss in the CWE group was significantly greater compared to the placebo group.

Also, the average number of pizza slices consumed after the twelve-week treatment period was significantly lower in the CWE group (4.7±1.0) compared to the placebo group (3.9±1.1), although the average appetite was not significantly different between the two treatment groups at baseline. Consequently, the CWE possibly has a positive effect on suppressing appetite and moderately reducing food intake, especially for carbohydrates.

4.3.5 Effect of CWE on Clinical and Biochemical Variables

The mean values (mean ± SD) of blood markers, including blood glucose, liver function and kidney function tests, lipid profile, and CBC tests in both groups were measured at baseline and after twelve weeks treatment, and the results are shown in **Table 4.5**. After twelve weeks of treatment, statistically significant differences were observed either within or between groups in any of the blood markers, except for the CBC test. After the intervention period, the RBC level showed a clinically significant rise, whereas the PDW showed a significant drop in the CWE group compared to the placebo group.

4.3.5.1 Effect of CWE on Blood Serum Glucose

During twelve weeks of treatment, the fasting blood sugar (FBS) level increased in both groups (3.95±20.2 mg/dL in the CWE group and 2.14±7.2 mg/dL in the placebo group). However, the differences were not significant either within or between the CWE group or the placebo group.

4.3.5.2 Effect of CWE on Liver Function

Liver function parameters, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and direct and total bilirubin were assessed at baseline and after the twelve-week intervention, and are summarized in **Table 4.5**. The average AST level showed a rise of 0.8±9.4 IU/L in the CWE group, and a reduction of 0.3±4.6 IU/L in the placebo group. Conversely, ALT level showed a reduction of 0.03±2.7 IU/L in the CWE group, and an increase of 0.2±5.4 IU/L in the placebo group. Also, the ALP level showed a reduction of 18.5±64.4 IU/L and 25.14±49.7 IU/L in both the CWE and the placebo group respectively. In addition, direct bilirubin showed a reduction of 0.03±0.1 mg/dL and 0.02±0.1 mg/dL in both the CWE group and the placebo group respectively. Similarly, a reduction of 0.09±0.3 mg/dL and 0.1±0.5 mg/dL was observed in the average total bilirubin in both the CWE group and the placebo group respectively. However, based on the paired and student t-tests, the changes in average values of any of the variables mentioned, were not significant either within or between any of the treatment groups.

4.3.5.3 Effect of CWE on Kidney Function

Kidney function parameters, including uric acid, urea, and creatinine, were measured at baseline and after the twelve-week intervention, and are presented in **Table 4.5**. In the CWE group, the uric acid level showed no visible changes during intervention, whereas in the placebo group a rise of 0.1±1.2 mg/dL was observed, which was not significant. The average urea level was decreased in both groups (-0.8±10.8 mg/dL in the CWE group and -0.5±6.7 mg/dL in the placebo group). Although the urea reduction in the CWE group was higher than in the placebo group, the differences in the reductions were not significant either within, or between, the two groups. Similarly, the mean creatinine levels showed a

reduction in both groups (-.0.1±0.2 mg/dL in the CWE and -0.1±0.2 mg/dL in the placebo groups), but the differences were not significant between the treatment groups.

4.3.5.4 Effect of CWE on Lipid Profile

Lipid profile variables, including serum total cholesterol (T-C), triglyceride (TG), HDL-cholesterol, LDL-cholesterol, LDL to HDL ratio (LDL/HDL), and cholesterol to HDL ratio (Chol/HDL) were measured at baseline and after twelve weeks of treatment, and are shown in **Table 4.5**. During the intervention period, the mean cholesterol values in the CWE group showed a reduction of -10.3±36.4 mg/dL, whereas the average cholesterol level in the placebo showed an increase of 7.1±39.1 mg/dL. However, the changes in average total cholesterol were not significant either within, or between, the CWE group and the placebo group.

Also, the triglyceride levels increased in both groups (11.6±48.8 mg/dL in the CWE group and 23.1±56.7 mg/dL in the placebo group) during the twelve-week study. The average triglyceride level in the placebo group was nearly doubled compared to the CWE group. However, the differences were not significant between two groups. In the CWE group, an increase of 0.8±12.0 mg/dL was observed in HDL level, whereas in the placebo group, a reduction of -1.2±7.9 mg/dL was detected. However, the differences between two groups during the treatment period were not significant. The LDL level increased in in both groups (1.8±41.8 mg/dL in the CWE group and 4.0±39.9 mg/dL in the placebo group), but the changes were not significant either within, or between, the CWE group and the placebo group. Also, the LDL to HDL ratio in the placebo group showed a rise of 0.1±0.9, whereas in the CWE group, no significant changes were observed. However, the differences were not significant either between or within any of the treatment groups.

Also, the cholesterol to HDL ratio showed reduction of 0.2±1.2 in the CWE group, but an increase of 0.2±1 in the placebo group. However, the differences which occurred during the twelve-week study were not significant between the CWE group and the placebo group.

4.3.5.5 Effect of CWE on Hematological Parameters (CBC)

The hematological parameters (complete blood cell count - CBC test) which were determined included WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, RDW-CV, PDW, and MPV. The mean values of these variables, assessed at baseline and after twelve weeks of treatment, are summarized in **Table 4.5**. After twelve weeks of treatment, the WBC levels increased in both groups (0.45±1.8 10^3/µL in the CWE group and 0.2±1.8 10^3/µL, in the placebo group). However, the changes in WBC level were not significant, either within or between the two groups. The RBC levels in the CWE group increased significantly during the intervention (0.3±0.3 10^6/µL), whereas in the placebo group, a reduction of 0.2±0.5 10^6/µL was observed, which was not significant. However, the differences in the mean RBC changes were significantly higher in the CWE group compared to the placebo group. Similarly, the average hemoglobin values were significantly increased in the CWE group (0.5±0.7 mg/dL), whereas the rise in the placebo group was not significant (0.4±1.0 mg/dL), and the differences were not significant between the two treatment groups after intervention period.

The mean HCT percentage increased in both groups (1.8% in the CWE group and 0.02%, in the placebo group). Again, no significant differences were observed in either of the treatment groups during the study. On the other hand, the MCV and MCH values were reduced in both groups (-0.6±4.3 fL in the CWE group and -0.2±10.2 fL, in the

placebo group for MCV; -0.3±1.7 pg in the CWE group and -0.5±2.2 pg, in the placebo group, for MCH). However, the changes were not significant either in the CWE group or in the placebo. Also, no significant differences were observed between the two groups after 12 weeks of treatment. However, the average MCHC values increase in both groups (0.3±1.1 g/dL in the CWE group and 0.18±1.01 g/dL in the placebo group); neither of the MCHC levels in the two groups was increased significantly. Also, the differences in increase were not significant between the CWE group and the placebo group.

After treatment, the PLT values showed an increase of 3.9±53.9 10^3/µL in the CWE group, and a decrease of 19.8±69.8 10^3/µL in the placebo group. However, the changes in PLT values were not significant, both within, and between, the two treatment groups. Similarly, the percentage of RDW showed an increase of 0.4% in the CWE group, but a reduction of -0.2% in the placebo group, and showing insignificant changes between and within the CWE group and the placebo group. In the CWE group a decrease of 1.8±3.1 fL was observed in the mean PDW, whereas in the placebo group, it showed an increase of 2.0±3.4 fL. However, the differences in PDW were significant between the two groups after the treatment period. Finally, the MPV values were increased in both groups (1.0±1.7 fL in the CWE group and 0.1±1.6 fL, in the placebo group) during the twelve-week treatment period, and were not significant in either of the treatment groups. Although the rise in the CWE group was much higher than in the placebo group, the differences were not significant between the two treatment groups.

4.3.5.6 Effect of CWE on Urine Biomarker (USG)

Urine-specific gravity (USG), as one of the urine markers, was measured at baseline and after the twelve-week treatment, and the mean values are shown in **Table 4.5**. During

the twelve-week intervention, the mean USG showed a reduction of 0.9±6.9 g/mL in the CWE group, whereas in the placebo group, an increase of 1.4±7.3 g/mL was observed. However, the differences were not significant either within or between CWE group and placebo.

4.3.6 Effect of CWE on Para-Clinical Variables

Para-clinical variables, including DBP, SBP, and HR, as markers of heart function, were measured at baseline and after twelve weeks of treatment, and the mean values are summarized in **Table 4.5**. In the CWE group, the SBP showed an increase of 0.7±5.4 mmHg after the treatment period, whereas no significant changes were observed in the placebo group. Also, the DBP showed an increase of 0.4±4.7 mmHg in the CWE group, and in the placebo group a decrease of 0.8±4.7 mmHg was observed. However, the differences were not significant either within or between treatment groups during the twelve-week intervention. The average HR showed a minor decrease in both groups (-0.6±4 bpm in the CWE and -0.8±3.8 bpm in the placebo group) after the twelve-week treatment. No statistically significant changes were observed in either of the treatment groups. Hence, consumption of CWE did not show any clinically significant effects on BP and HR as markers of the heart function.

4.3.7 Safety Issues and Adverse Events

According to the findings of this study, no clinically significant changes were observed for the clinical and para-clinical parameters between and within the two study groups during the twelve-week intervention. Also, no statistically significant effects were observed in the heart, liver and kidney functions between the groups (**Table 4.5**). Of the total of sixty subjects who completed the study, only the placebo participants experienced

skin allergy to the placebo product. Study also shows that there was no adverse events were reported during the physical examinations. Also, no adverse events were reported by any of the participants during the 12 weeks of CWE intervention.

Table 4.5: Changes (Mean ± SD) in clinical and para-clinical parameters between and within groups after twelve weeks intervention

Variables			Week 0 (baseline)		Week 12	
	Reference range		Placebo Group (n=29)	CWE group (n=31)	Placebo Group (n=29)	CWE group (n=31)
Blood serum assessments						
FBS	70-110	mg/dL	91.6 ± 7.4	91.2 ± 12.0	93.7 ± 3.4	95.2 ± 11.8
Liver function test						
AST, SGOT	5-40	IU/L	16.9 ± 4.5	16.8 ± 4.4	16.6 ± 1.6	17.6 ± 7.8
ALT, SGPT	5-40	IU/L	16.1 ± 4.8	16.6 ± 4.7	16.3 ± 5.9	16.3 ± 4.9
ALP	64-306	IU/L	173.1 ± 28.0	181.5 ± 36.6	148.0 ± 30.9	163.0 ± 46.3
Bilirubin, D	<0.25	mg/dL	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Bilirubin, T	0.2-1.1	mg/dL	1.1 ± 0.5	1.2 ± 0.6	1.0 ± 0.5	1.1 ± 0.6
Kidney function test						
Creatinine	0.4-1.5	mg/dL	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
UA	3-6	mg/dL	4.1 ± 1.1	4.2 ± 1.0	4.2 ± 0.7	4.2 ± 0.5
Urea	10-50	mg/dL	28.9 ± 7.0	29.6 ± 7.7	28.4 ± 3.3	28.9 ± 8.3
Lipid profile						
T-C	<200	mg/dL	183.3 ± 22.6	209.3 ± 29.9	190.4 ± 51.9	199.0 ± 25.1
TG	<150	mg/dL	121.9 ± 41.5	112.8 ± 35.1	145.0 ± 50.4	124.4 ± 42.6
HDL-C	>46	mg/dL	53.0 ± 9.9	55.9 ± 9.6	51.7 ± 7.7	56.7 ± 10.1
LDL-C	<130	mg/dL	106.7 ± 17.7	123.9 ± 28.7	110.8 ± 41.9	125.8 ± 25.9
LDL/HDL	<2.8		2.1 ± 0.5	2.3 ± 0.8	2.2 ± 0.9	2.3 ± 0.6
Chol/HDL	<4.3		3.5 ± 0.6	3.9 ± 1.1	3.8 ± 1.2	3.7 ± 1.0
CBC test						
WBC	4-10	103/µL	6.8 ± 1.6	6.4 ± 1.4	6.9 ± 1.4	6.9 ± 1.8
RBC	3.6-6.1	106/ µL	4.8 ± 0.5	4.5 ± 0.3	4.6 ± 0.3	4.7 ± 0.3*
HGB	11.5-18.8	(g/dL)	12.4 ± 0.9	12.8 ± 0.7	12.7 ± 0.6	13.3 ± 0.6 Ł
HCT	34-54	(%)	38.7 ± 3.0	39.0 ± 2.9	38.7 ± 2.4	40.8 ± 1.5
MCV	80-100	fL	84.7 ± 11.4	87.7 ± 4.2	84.6 ± 6.3	87.1 ± 5.9
MCH	27-36	(pg)	28.3 ± 2.1	28.8 ± 1.5	27.8 ± 1.8	28.5 ± 1.6
MCHC	32-36	(g/dL)	32.8 ± 1.1	32.6 ± 1.1	32.9 ± 0.3	32.8 ± 1.1
PLT	150-450	103/µL	272.5 ± 60.5	228.9 ± 40.3	252.7 ± 63.4	232.8 ± 52.7
RDW	11.6-14.6	(%)	13.3 ± 0.8	13.1 ± 1.2	13.1 ± 0.5	13.5 ± 1.0
PDW	7-20	fL	14.0 ± 3.3	13.8 ± 2.9	15.9 ± 0.9	12.0 ± 2.7*
MPV	6-13	fL	9.5 ± 1.1	8.7 ± 1.2	9.5 ± 1.2	9.7 ± 1.41
Urine test						
USG	1.015-1.025	g/mL	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.006
Vital signs						
DBP	60-90	mmHg	74.3 ± 6.0	75.5 ± 7.9	71.0 ± 7.6	75.9 ± 6.80
SBP	90-140	mmHg	111.3 ± 10.3	112.7 ± 10.4	111.3 ± 9.5	113.4 ± 11.21
HR	60-100	bpm	75.2 ± 8.7	78.1 ± 9.1	74.5 ± 8.6	77.5 ± 8.11

*, Significant difference from baseline compared to placebo ($p < 0.01$), independent samples t-test
Ł, Significant difference from baseline within group ($p < 0.01$), paired samples t-test

4.4 Detection of Phytochemicals Using GC-MS

The analytical results of the phytochemicals extracted into the caraway aqueous extract (CWE) are shown in the gas chromatogram (**Figure 4.2**). The predominant ingredients detected from the GC-MS analysis were a range of volatile and phenolic compounds, especially simple monoterpenes, including limonene, γ-terpinene, *trans*-carveol, carvone, carvacrol, and thymol.

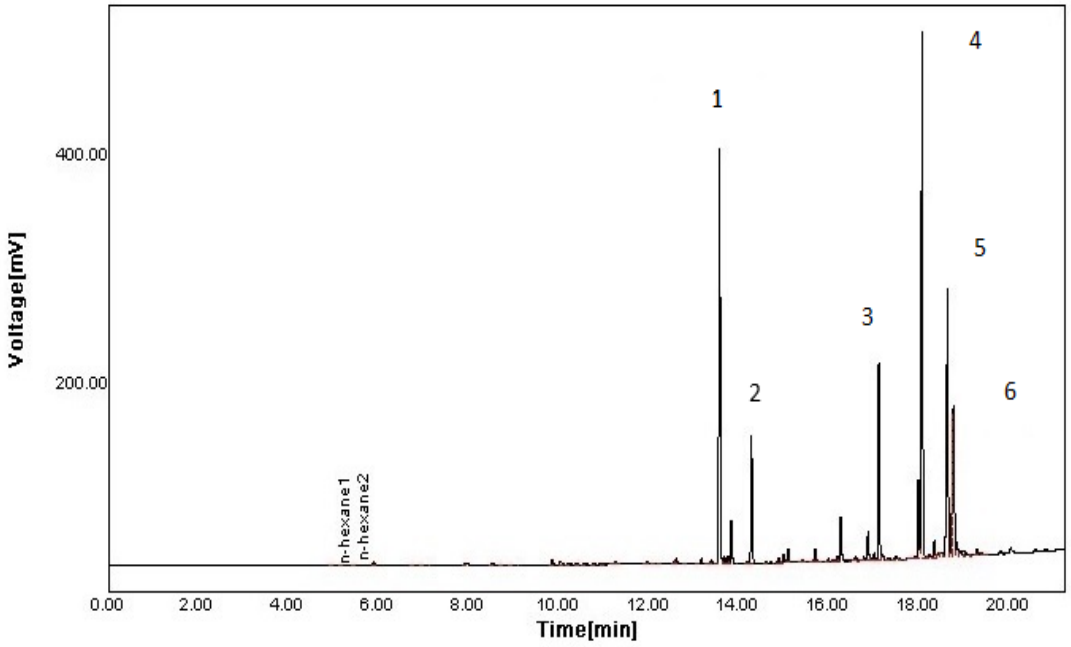


Figure 4.2: Chromatogram of CWE infusion extracted by HS-SPME

*CWE volatiles obtained by steam distillation with subsequent hexane extraction include: 1: limonene, 2: γ-terpinene, 3: trans-carveol, 4: carvone, 5: thymol, 6:carvacrol.

CHAPTER 5: DISCUSSION

5.1 An Overview

Based on the findings mentioned in the previous chapter, the results will be discussed in this section based on the objectives of this study. The discussion will be on examining the effect of CWE intake on body composition, anthropometric indices and appetite in overweight and obese women, investigating the safety of caraway intake for human, and analysis of the phytochemicals present in CWE which will be explained in details respectively.

5.2 Efficacy Evaluation of CWE

5.2.1 Effect of CWE on Body Composition, and Anthropometric Indices

The initial objective of this study was to study the effect of CWE intake on body composition, anthropometric indices and appetite in overweight and obese women. Hence, the weight-lowering activity of caraway as an established traditional medicinal plant in Iran was studied in a triple-blind, placebo-controlled, clinical trial in Iranian overweight and obese females. As nutrition and exercise are the two major components of life-style in the control of body weight, participants were enrolled who were habitually performing aerobics during the whole period of the intervention, without changing their dietary habits and lifestyle patterns.

This study showed that consumption of 30 ml/day CWE may result in reasonable anti-obesity effects. Similar findings were observed in a recent animal study reporting that 0.1% carvacrol, as one the major constituents of caraway, can prevent obesity and induce weight and fat loss in mice fed with high fat diet (Cho *et al.*, 2012). Also, several animal

studies in normal and diabetic rats have proved the beneficial effects of caraway aqueous extract (20 mg/kg) on treating a number of health problems such as hyperglycemia (Eddouks *et al.*, 2004), and hyperlipidemia (Lemhadri *et al.*, 2006) which are recognized as common health consequences of obesity. Together, this clinical trial suggests a possible phytotherapeutic approach and natural medication for the application of CWE in the management of obesity.

In this study, changes in body composition, and anthropometric indices in healthy, overweight, and obese females after the twelve-week consumption of CWE or placebo were evaluated. Clinically significant improvement in all the stated parameters after the treatment period was observed. Among the body composition variables, total body weight and fat percentage showed significant reduction in participants consuming CWE. These findings support the study hypothesis that consumption of caraway seed extract could be helpful in reducing weight and fat percentage in humans.

Based on the findings of GC-MS analysis of CWE in this study, the main detected ingredients were of different volatile and phenolic compounds, including limonene, terpinene, carveol, carvone, carvacrol, and thymol. This is in line with a previous study, reporting that body weight and fat loss might be linked to anti-oxidant, anti-inflammatory, and anti-bacterial properties of caraway phytochemicals, especially phenolic compounds, such as carvacrol, and the unsaturated fatty acids (UFA) (Laribi *et al.*, 2013). The intake of such bioactive ingredients with high antimicrobial activity, prevents the proliferation of pathogenic microorganisms, and thus enhances the growth and multiplying of beneficial gut bacteria (Hawrelak *et al.*, 2009; Iacobellis *et al.*, 2005). These interactions will possibly improve the balance of gut microbial ecology and alter gastrointestinal (GI) microbiome towards higher beneficial gut bacteria which could further improve digestion

and absorption of the ingested food providing homeostasis in GI tract (Can Baser, 2008; Michiels *et al.*, 2007; Upreti *et al.*, 2008). On the other hand, gut microflora (GM) could affect the host metabolism through regulating the expression of human genes. Hence, the interplay between GM, gene expression, and the host metabolic activity may have an effect on obesity and weight changes. This assumption is in line with the recent findings demonstrating that GM composition could have mutual interplay with the host, programming and affecting metabolism in human body altering body composition (Backhed, 2011; Musso *et al.*, 2011). The suggested mechanism of action is explained in **Figure 5.1**.

In this procedure, the gut microflora, which were altered following the antimicrobial actions of the present bioactive compounds, such as phenolic compounds, may induce the expression of specific genes involved in fat metabolism, which consequently leads to constraining inflammation in adipose tissue, and preventing adipogenesis (Cho *et al.*, 2012; Lombardo & Chicco, 2006). Previous studies indicated that the balanced gut microbiota prevents macrophage infiltration into adipose tissue causing interruption in the transformation of pre-adipocytes to mature adipocytes. This procedure will subsequently lead to constraint in adipogenesis and differentiation of adipocytes (Cani & Delzenne, 2011). These illustrations reinforce the possible role of the anti-inflammatory properties of the compounds present in CWE in weight and fat loss. Also, this hypothesis is in line with the findings of recent studies reporting that inflammation is related to body fat (Wesseltoft-Rao *et al.*, 2012). Hence reducing the fat mass, which occurred in this study, might also be linked with anti-inflammatory reactions occurring in the human body.

Furthermore, UFAs could induce fatty acid oxidation resulting in lipolysis and fat loss (Iyer *et al.*, 2010; Kalupahana *et al.*, 2011). In addition, caraway essential oils and phenolic compounds could inhibit lipid peroxidation and enhance apoptosis in pre-adipocytes due to their antioxidant properties (Samojlik *et al.*, 2010). These compounds can diminish adipose tissue and body fat mass through inhibiting adipogenesis, inducing apoptosis in pre-adipocytes, and stimulating lipolysis in adipocytes (Hsu & Yen, 2007b; Hsu & Yen, 2008; Rayalam *et al.*, 2008). In addition, previous studies have proposed that an aqueous extract of caraway could display lipolytic activity due to reducing intestinal lipid absorption, by binding with bile acids. A similar mechanism might be appropriate to describe the observed fat lowering activity of CWE. Also, caraway may facilitate weight and fat loss through inhibiting lipid biosynthesis by altering lipid metabolism which may contribute to the regulation of body fat (Lemhadri *et al.*, 2006). Accordingly, the weight-lowering property of caraway may be mediated by the improvement of digestion, absorption, and lipid metabolism. In conclusion, this trial has revealed that daily consumption of CWE (30 ml/day) for twelve weeks evokes an advantageous effect on overweight and obesity. This outcome supports its consumption by the Iranian population for the treatment and management of obesity.

With regard to the body composition changes, body weight and fat were decreased significantly, and are consistent with a recent animal study demonstrating the potent anti-obesity activity of carvacrol in obese mice (Cho *et al.*, 2012). In contrast, no significant alterations were observed in the body water percentage in participants during the study. These findings show that the intake of CWE should not have any adverse effects on body health. Usually, the percentage of water will be changed during various disease states, and assessing body water is essential in determining the occurrence and progression of diseases (Glenda Winson, 2001).

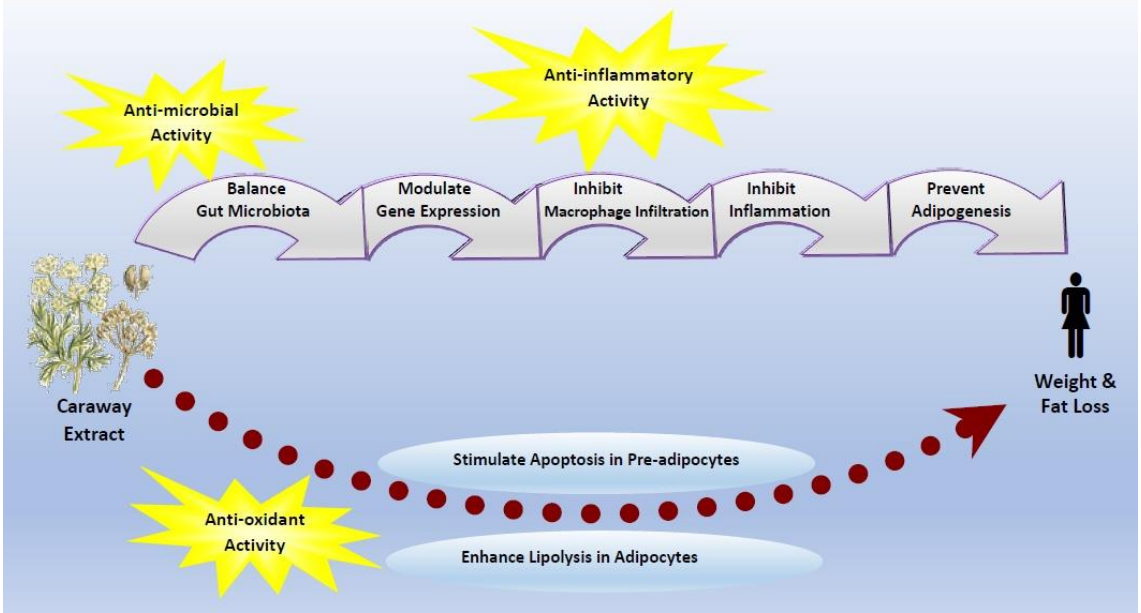


Figure 5.1: Possible CWE metabolic actions on the human body during weight loss

Results on body muscle percentage showed an improvement in the CWE group, whereas there were no significant changes in muscle percentage in the placebo group during the course of the study. Thus physical activity and aerobic exercises did not have an interfering effect on decreasing body weight and fat percentage of the participants. Accordingly, these outcomes suggest that the positive alterations in body composition were probably related to the phytochemicals present in CWE, and not essentially with the exercise, even though it was expected that physical activity would probably have a synergistic effect on lowering body weight and body fat in those study participants consuming CWE. Furthermore, the bio-products that have been formed during lipolysis were probably transformed into muscle induced by exercise, synergistically, and the loss in body fat and rise in muscle mass is most likely due to physiological adaptations to physical activity (Nissen & Sharp, 2003; Panton *et al.*, 2000; Pittler & Ernst, 2004).

With regard to the alterations in body size, as expected, all anthropometric indices showed a significant decrease in those study participants consuming CWE compared to

the placebo group. The findings show that the waist circumference, the waist to hip ratio, the thigh circumference, and the MUAC decreased significantly after the twelve-week consumption of CWE. According to the demographic values of this study, all of the participants had WC greater than borderline, showing abdominal obesity at baseline. The WHR and WC are the major determinants and markers of central adiposity, and are recognized as the major risk factors for the health related issues, such as cardiovascular problems, ischemic stroke, cancer mortality, myocardial infarction, diabetes, impaired fibrinolytic activity, etc. (Dagenais *et al.*, 2005; Landin *et al.*, 1990; Lee *et al.*, 2008; Suk *et al.*, 2003; Wang *et al.*, 2005; Zhang *et al.*, 2008). Consequently, reducing WHR and WC through consumption of CWE may be of value in the prevention and treatment of abdominal obesity and its complications. Moreover, waist circumference, reflects the content within the abdominal cavity as well as visceral fat and subcutaneous fat (Pouliot *et al.*, 1994). Flatus and faecal content of the gut can affect waist circumference. The WC reduction in the CWE was 6.2 cm yet the total weight reduction was only 1.9 kg. There is very roughly a relationship between WC and body weight of approximately 1 cm WC reduction per 1 kg weight reduction. CWE is a carminative, relaxing the gut. It is plausible, that the larger than expected reduction of WC reflects an effect of CWE on flushing the large colon. This is in line with the reports of the CWE subjects regarding improvements in their bowel function and their bloating and flatulence problems.

On the other hand, the body size values did not show any significant reduction in the placebo group during the course of the study, except for the thigh circumference and the MUAC, which indicates that exercise did not have any interfering or synergistic effect on the reduction of abdominal obesity and visceral fat.

Anthropometric indices are known as beneficial indicators for evaluating the adiposity of an individual, and worthy markers of the health and nutritional status of an individual (Mandal *et al.*, 2011). In this study, the results of the anthropometric assessments are in line with the outcomes of body composition measures, indicating that there is a link between a reduction in body size, body fat, and weight, as lowering the WHR and WC reflects a reduction in visceral fat. Also, these results show a possible contribution of CWE in lowering abdominal fat. These findings indicate that the balancing, improvement, and normalization in body size and body composition have occurred simultaneously. Together, these results provide complementary and supplementary data which support the study hypothesis regarding the potential anti-obesity activity of caraway aqueous extract ingredients for human.

5.2.2 Effect of CWE on Food Intake, and Appetite

With regard to the food intake assessments during the study intervention, the intake of calories and all macronutrients, including carbohydrates, proteins, and total fat, showed a significant decrease in those subjects consuming CWE, but not in the placebo group. However, a significant difference in reduction between the CWE group and the placebo group was observed only in CHO intake. These findings are in line with the results of the appetite test which showed that the appetite level was reduced significantly in those subjects consuming CWE compared to the placebo group. In addition, the results of the *ad libitum* pizza test after completion of the study support these findings, indicating that the subjects in the CWE group consumed significantly fewer pizza slices compared to the placebo group showing that participants who consumed CWE during the twelve-week study period, had a lower appetite compared to those who consumed placebo although

there was no significant difference in the average level of appetite between two groups at baseline.

The appetite-suppressant effect of CWE is in agreement with the previous studies acknowledging caraway oil/aqueous extract to have appetite-regulating activity which might be linked to its carminative properties, and soothing effects on gastric contractions (Blumenthal, 1999; Reynolds, 1996; Sadowska & Obidoska, 2003). On the other hand, the findings of reducing food and CHO intake are in agreement with the changes in body composition which occurred during this study. During this intervention, the reduction in food intake following consumption of CWE did not have a negative effect on the body composition or nutritional status of the subjects, rather an improvement in body composition was observed. From these findings, it is possible that a moderate reduction in food intake and of CHO in particular, followed by consumption of CWE would induce weight loss, thereby lowering body size, excess body fat, and abdominal obesity, with no adverse effect on protein stores and waste in muscle mass.

As mentioned above, in this study, muscle mass gain was observed in the CWE group. On the other hand, clinical validations obtained from this study presented no adverse effects in the health status of subjects. These findings are in agreement with previous studies reporting that moderate restriction in calorie and CHO intake would have a potent beneficial effect on obesity, inflammation, hypertension, cardiovascular problems, diabetes mellitus, slow aging, cancer, delay disease onset, and extend lifespan, whereas most of the uncontrolled dietary restriction regimens might induce malnutrition and health problems, especially taken for a long-term period (Cava & Fontana, 2013; Colman *et al.*, 2009; Meckling *et al.*, 2004; Omodei *et al.*, 2013; Ritz & Gardner, 2006; Trepanowski *et al.*, 2011).

Accordingly, the reduction in food intake which was observed in this study did not show any adverse effect on the nutritional status and body composition of the participants. These results are consistent with the demonstrations of previous studies indicating that body composition and anthropometric indices are beneficial indicators reflecting the health and nutritional status of an individual (Glenda Winson, 2001; Mandal *et al.*, 2011). Such outcomes suggest that CWE is able to suppress appetite, and lower food intake with no depletion in protein stores and waste in muscle mass.

5.3 Safety Evaluation of CWE

5.3.1 Effect of CWE on Clinical and Para-Clinical Variables and Safety Issues

The second objective of this study was to examine the safety of caraway water extract (CWE) intake for humans. There appears to be no triple-blind clinical study on the safety and tolerability of any caraway preparations taken alone. A few studies have examined the safety of caraway oil and as a combination with other phytochemicals, but not as a single aqueous extract preparation (Madisch *et al.*, 2004; Westphal *et al.*, 1996). This study illustrates that daily consumption of CWE (30 ml/day) has a potential to reduce weight with no clinically significant adverse events or serious side effects. During the clinical trial, the intake of CWE did not show any side effects on major vital organ functions, including the heart, liver, and kidney. Also, no clinically significant changes were observed on urine-specific gravity, serum blood glucose level, and lipid profile. As a conclusion, no significant adverse events were observed in the clinical and para-clinical valuations with either treatment during the twelve-week intervention period, and the CWE was well-tolerated by all of the participants. These findings may support other opinions which have recommended caraway as a safe natural product with several

therapeutic effects for humans (May *et al.*, 2000; Sadowska & Obidoska, 2003; Samojlik *et al.*, 2010; Westphal *et al.*, 1996).

Caraway and its by-products have a long history of folk usage, and are widely-used plant-based products (Guarrera & Savo, 2013; Halberstein, 2005). There are a number of in vitro and in vivo studies which report different therapeutic potentials and biological activities for caraway oil/aqueous extract and its derivatives, for example anti-microbial, anti-inflammatory, antioxidant, anti-cancer, and anti-spasmodic effects (Alhaider *et al.*, 2006; Can Baser, 2008; Johri, 2011). However, studies on the safety and tolerability of caraway seed and its extracts in humans are few. In this research, the safety and tolerability of CWE were studied in healthy, overweight and obese women in a randomized, triple-blind, placebo-controlled clinical trial during twelve weeks. After the treatment period, no severe adverse events were observed in any of the participants with respect to the cardiovascular, hepatic, renal, or hemopoietic systems. Also, the results revealed no significant changes in the clinical and para-clinical variables. As a result, this CWE may be regarded as being well-tolerated, and safe to consume with no problematic effects for humans. The findings concerning the adverse effects of CWE intake were as expected, and in agreement with those of the earlier studies, indicating that there are no known and recognized cautions, adverse events, or drug interactions associated with the consumption of caraway (Blumenthal, 1999; Capasso *et al.*, 2003).

During the twelve-week of treatment, there were no significant changes in the average values of any of the biomarkers, except in the hematological variables showing an increase in RBC and a decrease in PDW. Previous studies have recommended caraway oil or aqueous extract and its derivatives as a potent plant, with anti-hyperglycemic and anti-hyperlipidemic activities lowering blood glucose, cholesterol, LDL, and TG levels

which could be useful in treating diabetes and hyperlipidemia (Eddouks *et al.*, 2004; Ene *et al.*, 2007; Haidari *et al.*, 2011; Lemhadri *et al.*, 2006). The measurements of lipid profile in this study showed a slight drop in the total cholesterol level in the CWE group, and a slight rise in the placebo group without significantly affecting TG, and LDL and HDL cholesterol. The lack of significance may be associated with a number of factors. One reason might be due to the small sample size. In this case, allotting a sufficiently large sample size might be more effective and helpful to detect substantial change. Furthermore, the study population allotted were mainly comprised of subjects with normal levels of serum blood glucose and lipid profile at baseline, whereas the reported hypolipidemic and hypoglycemic activity of caraway oil or aqueous extract and its derivatives were shown in diabetic rats or in animals suffering from hyperlipidemia (Eddouks *et al.*, 2004; Ene *et al.*, 2007; Haidari *et al.*, 2011; Lemhadri *et al.*, 2006). An alternative explanation for the lack of significant effect of CWE on lipid profile and glucose level in this study might be associated with the dosage and composition of caraway product applied. Hence, further studies are recommended to assess the hypoglycemic and hypolipidemic activity of different caraway products at different dosages, and also through the administration of caraway oil containing more bioactive and volatile compounds which might result in more biologically diverse findings.

With respect to the heart function test, no clinically significant changes were observed in the blood pressure or heart rate in any of the treatment groups. Such outcomes differ from previous findings wherein caraway seeds have been used traditionally as folk remedy to treat hypertension and decrease blood pressure (Tahraoui *et al.*, 2007). An explanation of this might be due to the fact that the antihypertensive activity of caraway has only been reported ethnomedically, and has not been examined scientifically. The

blood-pressure-lowering effect of CWE should be examined in an animal hypertensive model initially to assess perceptible results.

The kidney function test showed that usage of CWE would not have any untoward effects on kidney function. These outcomes are in line with the findings of previous studies demonstrating the reno-protective property of caraway aqueous extract (60 mg/kg) in rodents (Sadiq *et al.*, 2010). Also, after the twelve-week treatment period with CWE, no adverse events were shown in liver function biomarkers. These results are consistent with the reports supporting hepatoprotective potential of caraway oil extract (minimum 0.13 μM) administration in mice (Naderi-Kalali *et al.,* 2005; Samojlik *et al.*, 2010).

The findings of the blood CBC test indicated that the average level of RBC improved significantly while the mean values of PDW showed a significant reduction in subjects consuming CWE as compared with the placebo group after the twelve-week intervention. On the other hand, HGB levels showed a significant increase only in the CWE group, which supports the findings related to the significant rise observed in the RBC levels. These alterations display the potential valuable influence of CWE as a phytotherapeutic agent for the treatment of anemia. It is possible that treatment with CWE may well compensate for the low RBC level in patients suffering from anemia. These judgments are in line with an earlier animal study which suggested that caraway water extract (2.5 g/100 ml) may have protective properties against anemia through stimulating the absorption of iron in the gastrointestinal tract (El-Shobaki *et al.*, 1990). Also, the reduction in the PDW levels in patients consuming CWE is in line with a previous study which reported the anti-platelet properties of carvacrol (Can Baser, 2008). Prior studies revealed that hyperthyroidism is linked to a rise in MPV (mean platelet volume) levels

and a decline in PDW levels (Ford & Carter, 1990). In addition, administration of a hydro-alcoholic extract of caraway (1600 mg/kg) displayed a hyperthyroidism influence through escalation in T3 and T4 levels, and a reduction in TSH levels (Dehghani *et al.*, 2010). Also, the weight of rats in the caraway extract group was remarkably lower than the control group, which shows the weight lowering effect of caraway extract.

Thyroid hormones are recognized as determining factors of the basal metabolic rate and energy expenditure. Therefore, modifying thyroid hormone levels affects thermogenesis in the body leading to major changes in body composition, reducing body fat, body weight, and appetite (Johnstone *et al.*, 2005; Kim, 2008; Moreno *et al.*, 2008). In line with these findings, the present outcomes propose a moderate anti-hypothyroidism influence of CWE in humans. This plant preparation may influence hypothyroidism homeostasis, which could possibly give rise to an enhanced metabolic rate and, in consequence, a reduction of body weight and fat percentage. This is in agreement with the body composition and appetite changes observed in this study. The probable mechanism of action and effect of consumption of CWE on biochemical variables are shown in **Figure 5.2**. As a conclusion, this study suggests that CWE, as a potential anti-obesity plant, can be consumed in moderation and regularly as part of a healthy, balanced diet for normalizing body composition and appetite without any severe adverse effects.

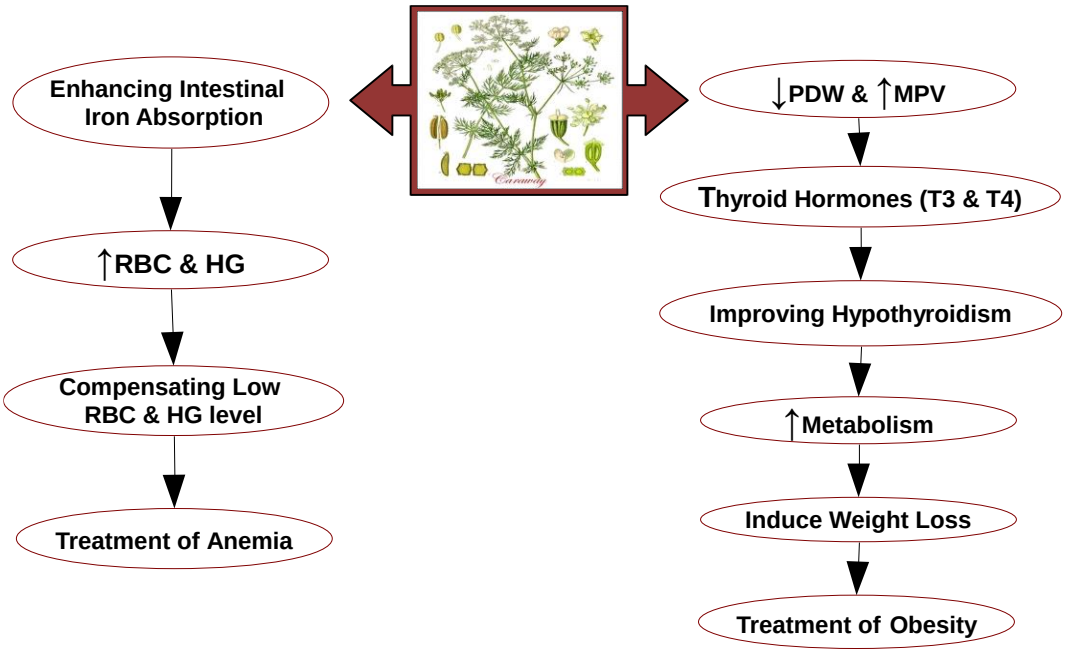


Figure 5.2: Possible therapeutic effects of CWE on the human body

5.4 Analysis of CWE Phytochemicals

The third objective of this study was to identify the major phytochemical constituents present in the steam-distilled caraway extract using GC-MS analysis. The results show that most of the constituents detected from the GC-MS analysis were a range of different volatile and phenolic compounds, especially simple monoterpenes, including limonene, γ-terpinene, *trans*-carveol, carvone, carvacrol, and thymol, and the phenylpropene derivative, anethole. The results of the GC-MS analysis in this study show that these findings are consistent with the major compounds characterized from the caraway extract as reported in previous studies (Iacobellis *et al.*, 2005; Laribi *et al.*, 2013; Park *et al.*, 2008; Razzaghi-Abyaneh *et al.*, 2009; Richter & Schellenberg, 2007; Rivera *et al.*, 2010; Samojlik *et al.*, 2010; Seo *et al.*, 2009; Simic *et al.*, 2008). As stated in the previous sections, a number of evidences show the relation of these phytochemicals with obesity and weight loss which are in line with the findings of this study.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 An Overview

In Islamic traditional medicine, there are numerous potential plants recommended for treatment of different health problems such as obesity. This research was carried out to examine the efficacy and safety of a caraway extract, as an example of a traditional, anti-obesity medicinal plant, on healthy obese and overweight women in a randomized, triple blind, placebo controlled, clinical trial during a twelve-week period. The results showed that consumption of CWE could be of practical value in the management of obesity.

According to the previous studies, anti-obesity plants are able to induce weight loss through four different mechanisms, including suppressing appetite, inducing lipolysis, inhibiting lipogenesis, and enhancing metabolism. In addition, some of the potent anti-obesity plants can induce weight loss through two or more mechanisms (Yun, 2010). From the findings of this study, CWE can induce weight loss through all the four mechanisms stated earlier.

Also, it can be concluded that the anti-obesity properties of CWE are due to the presence of the bioactive ingredients. As mentioned previously, the phytochemicals extracted from the CWE, as detected by GC-MS, were well-known volatile compounds, including limonene, γ-terpinene, trans-carveol, carvone, thymol, and carvacrol. These bioactive compounds present in CWE may be effective in the management of obesity, reducing body size, body weight, body fat and appetite most likely through a combination of four major bioactivities including anti-microbial, anti-oxidant, and anti-inflammatory properties, together with appetite-suppressing activity.

This study is the first triple-blind clinical trial examining the effects of consumption of CWE on body composition and anthropometric indices, along with a physical activity program, and investigating the anti-obesity effect of CWE in overweight and obese females during twelve weeks of treatment. Moreover, this study has four additional strengths listed as follows:

Firstly, participants consuming 30 ml/day CWE for 12 weeks showed significant reductions in body weight, body fat, and also body size, as compared with the placebo group, although they did not change their dietary habits. Secondly, oral administration of CWE results in lowering CHO intake and appetite, which would induce weight loss, along with improving body composition and blood biomarkers with no problematic effects on body health. Thirdly, this interventional study was a randomized triple-blinded placebo-controlled clinical trial which improves the precision and accuracy of the outcomes and decreases probable bias in the results. Fourthly, this is the first evidenced-based study demonstrating the safety and tolerability of a caraway extract in humans. Treatment with CWE was on the whole, well-tolerated with no informed adverse effects. In addition, the significant rise in RBC and reduction in PDW afforded by the twelve-week administration of the caraway extract suggests a potential use of this plant-derived extract in the management of anemia and hypothyroidism, respectively, which requires further investigation.

6.2 Implications and Recommendations

In this section, the researcher summarizes the findings of all chapters and suggests the future direction for research. The recommendations which are acknowledged are listed as follows:

Firstly, parameters assessed in this study were only limited to blood, para-clinical and clinical variable. However, for studying the underlying mechanism of action, supplementary assessments such as hormones or other biomarkers which might play role in treating obesity are required to be done. Also, for verifying and evidencing the metabolisms proposed in this study, examining probable alterations in fecal microflora, also possible changes at molecular level and determining the genes involved in these changes are suggested.

Secondly, findings obtained in this trial are restricted to an aqueous extract of caraway, and so additional estimates are recommended to examine the anti-obesity influence of different type of caraway seed extracts such as caraway oil extract achieved from other procedures or preparation methods. Also, studying the effects of caraway by-products or bioactive compounds are suggested to identify the major phytochemicals displaying different bioactivities. Moreover, only a single dose of CWE was examined in this clinical trial. Hence, additional studies are recommended to examine the safety and efficacy of CWE in different doses through conducting multiple dosage to achieve more precise dosing limitations.

Thirdly, only healthy Iranian adult female who were overweight and obese were eligible to participate in this trial and volunteers with medical complications have been excluded from the study. As previous studies have identified several therapeutic properties of caraway and its derivatives, it would be much valued and recommended to examine the weigh lowering activity of caraway in subjects suffering from other health problems such as hormonal dysfunction, hypertension, hyperlipidemia and cardiovascular problems, hyperglycemia and diabetes, metabolic syndrome and other health problems which are mostly linked to obesity. Also, the study was conducted only in Iranian women

living in province of Yazd. Hence, it is proposed to conduct this study in other countries with different races, cultures and nationalities wherein the outcomes would be of higher value if compared with each other. On the other hand, the study population were in a limited age range. So, further studies are required to examine the efficacy and tolerability of CWE in other stage of life especially in children and elder population which might have lower immune system.

The other issue is concerned to the size of the study population. Although the sample size in this trial was statistically enough to achieve the feasible results, it is suggested to perform this study in a larger population to attain more accurate data. Besides, since this trial was examined only in adult females, it is recommended to study the weight lowering activity of CWE in males as well in order to achieve more reliable data by having homogenous population containing both genders. Finally, as exercise might induce an interfering or synergistic influence on weight and fat loss (Ross *et al.*, 2000), repeating this trial on individuals without physical activity is suggested.

This study also implies that intake of caraway could be useful in decreasing the complications of obesity, such as diabetes mellitus and cardiovascular diseases. However, additional studies, with different doses and preparations of CWE in other national and ethnic groups, with multiple genders, with larger population, in wider age groups, with a variety of health problems, and over a longer extended period of time, are recommended to determine more accurate data about the safety, tolerability, efficacy, and applicability of CWE, and to further improve the importance and worth of these primary clinical findings.

From the evidence presented in this study and in previous researches, it is hypothesized that the anti-obesity activity might be from the prebiotic effect of CWE in the gastrointestinal tract through modulating gut microflora. The mechanism of action of these phytochemical constituents needs to be defined at the molecular level. Altogether, the health benefits of CWE lay in the potential contribution of this plant-based product to the overall intake of potent phytochemicals, especially phenolic and volatile compounds. However, supplementary studies are required to examine the effects of these constituents in treating obesity and overweight at the molecular level. Also, the actual sites of this activity, the detailed mechanisms of action, and the specific bioactive components of caraway involved should be determined. The significant differences in RBC and PDW have no sinister meaning as they are well within the normal range. However, the rise of haemoglobin and RBC are indeed interesting and merit further investigation.

At the same time, efforts to make available this evidence and pursue the expanded human use of this traditional medicinal plant, and develop the consumption of caraway and its derivatives as a sustainable dietary practice, together with exercise, as a part of a healthy lifestyle, should be continued. It is hoped that the findings of this study will be useful for the management and fight against obesity and overweight using natural sources with potent anti-obesity activity to improve the quality of life of the world's population.

Overall, the research findings are important to enrich the knowledge and practices of Muslim heritage in traditional and complementary medicine with scientific values and empirical facts. It is recommended to study other potential herbal compounds and medicinal plants acclaimed in Islamic traditional references and disclose their probable health benefits, and their safety and efficacy, using modern scientific approaches.

REFERENCES

Abubakari, A.-R., Lauder, W., Agyemang, C., Jones, M., Kirk, A., & Bhopal, R. (2008). Prevalence and time trends in obesity among adult West African populations: A meta-analysis. *Obesity Reviews, 9*(4), 297-311.

Adams, R. P. (Ed.). (2007). *Identification of essential oil components by gas chromatography/mass spectrometry* (4 ed.). Carol Stream, IL, USA: Allured Publishing Corporation.

Adult Treatment Panel III. (2001). Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults. *American Medical Association, 285*(19), 2486-2497.

Ahn, J., Lee, H., Kim, S., & Ha, T. (2010). Curcumin-induced suppression of adipogenic differentiation is accompanied by activation of Wnt/β-catenin signaling. *American Journal of Physiology-Cell Physiology, 298*(6), C1510-C1516.

Al-Bataina, B. A., Maslat, A. O., & Al-Kofahi, M. M. (2003). Element analysis and biological studies on ten oriental spices using XRF and Ames test. *Journal of Trace Elements in Medicine and Biology, 17*(2), 85-90.

Al-Essa, M. K., Shafagoj, Y. A., Mohammed, F. I., & Afifi, F. U. (2010). Relaxant effect of ethanol extract of *Carum carvi* on dispersed intestinal smooth muscle cells of the guinea pig. *Pharmaceutical biology, 48*(1), 76-80. doi: 10.3109/13880200903046161

Alhaider, A., Al-Mofleh, I., Mossa, J., Al-Sohaibani, M., Rafatullah, S., & Qureshi, S. (2006). Effect of *Carum carvi* on experimentally induced gastric mucosal damage in wistar albino rats. *International Journal of Pharmacology, 2*(3), 309-315.

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W., & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry, 20*(4), 470-475.

Almoosawi, S., Fyfe, L., Ho, C., & Al-Dujaili, E. (2010). The effect of polyphenol-rich dark chocolate on fasting capillary whole blood glucose, total cholesterol, blood pressure and glucocorticoids in healthy overweight and obese subjects. *British Journal of Nutrition, 103*(06), 842-850.

Ambati, S., Yang, J. Y., Rayalam, S., Park, H. J., Della-Fera, M. A., & Baile, C. A. (2009). Ajoene exerts potent effects in 3T3-L1 adipocytes by inhibiting adipogenesis and inducing apoptosis. *Phytotherapy Research, 23*(4), 513-518.

Angelakis, E., Armougom, F., Million, M., & Raoult, D. (2012). The relationship between gut microbiota and weight gain in humans. *Future Microbiology, 7*(1), 91-109.

Aqili Khorasani, M. H. (2001). *Makhzan al-adviyah (The Storehouse of Medicaments)*. Research Institute for Islamic and Complementary Medicine, Iran University of Medical Sciences, Tehran, Iran: Bavardaran Press.

Arbo, M. D., Schmitt, G. C., Limberger, M. F., Charão, M. F., Moro, Â. M., Ribeiro, G. L., . . . Limberger, R. P. (2009). Subchronic toxicity of *Citrus aurantium* L. (Rutaceae) extract and *p*-synephrine in mice. *Regulatory Toxicology and Pharmacology, 54*(2), 114-117. doi: 10.1016/j.yrtph.2009.03.001

Armougom, F., Henry, M., Vialettes, B., Raccah, D., & Raoult, D. (2009). Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. *PLoS One, 4*(9), e7125.

Asghari, G., Rezazadeh, A., Hosseini-Esfahani, F., Mehrabi, Y., Mirmiran, P., & Azizi, F. (2012). Reliability, comparative validity and stability of dietary patterns derived from an FFQ in the Tehran Lipid and Glucose Study. *British Journal of Nutrition, 108*(06), 1109-1117.

Association of the European Self-Medication Industry-AESGP. (2010). *Legal and Regulatory Framework for Herbal Medicines.* from http://www.self-medication.org/publications/countryProfiles.asp

Auld, M. C., & Powell, L. M. (2009). Economics of food energy density and adolescent body weight. *Economica, 76*(304), 719-740. doi: 10.1111/j.1468-0335.2008.00709.x

Azaizeh, H., Saad, B., Cooper, E., & Said, O. (2010). Traditional Arabic and Islamic medicine, a re-emerging health aid. *Evidence-Based Complementary and Alternative Medicine, 7*(4), 419-424.

Backhed, F. (2011). Programming of host metabolism by the gut microbiota. *Annals of Nutrition and Metabolism, 58*, 44-52. doi: 10.1159/000328042

Bai, N., He, K., Roller, M., Zheng, B., Chen, X., Shao, Z., . . . Zheng, Q. (2008). Active compounds from *Lagerstroemia speciosa*, insulin-like glucose uptake-stimulatory/inhibitory and adipocyte differentiation-inhibitory activities in 3T3-L1 cells. *Journal of Agricultural and Food Chemistry, 56*(24), 11668-11674.

Balch, P. A. (2002). Herbal prescription for common health problems: Indigestion *Prescription for herbal healing: An easy-to-use A-Z reference to hundreds of common disorders and their herbal remedies* (pp. 537). Torquay, UK: Avery: Penguin

Balsiger, B. M., Murr, M. M., Poggio, J. L., & Sarr, M. G. (2000). Bariatric surgery: Surgery for weight control in patients with morbid obesity. *Medical Clinics of North America, 84*(2), 477-489.

Barakat Abu-Rmailah, B., & Afifi, F. (2000). Treatment with medicinal plants in Jordan. *Dirasat, Medical and Biological Sciences, 27*(1), 53-74.

Barker, P. E., Wagner, P. D., Stein, S. E., Bunk, D. M., Srivastava, S., & Omenn, G. S. (2006). Standards for plasma and serum proteomics in early cancer detection: A needs assessment report from the national institute of standards and technology-national cancer institute standards, methods, assays, reagents and technologies workshop, August 18–19, 2005. *Clinical Chemistry, 52*(9), 1669-1674.

Barnes, P. M., Bloom, B., Nahin, R. L., & Statistics, N. C. f. H. (2008). Complementary and alternative medicine use among adults and children: United States, 2007 *National Health Statistics Reports, No. 12*. Hyattsville, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics.

Barness, L. A., Opitz, J. M., & Gilbert-Barness, E. (2007). Obesity: Genetic, molecular, and environmental aspects. *American Journal of Medical Genetics. Part A, 143*(24), 3016-3034.

Bastard, J.-P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H., . . . Feve, B. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European Cytokine Network, 17*(1), 4-12.

Bennett, B. C. (2010). Economic botany: Twenty-five economically important plant families. *Encyclopedia of life support systems*. Oxford, UK: UNESCO-EOLSS Publishers.

Bergmeyer, H., Hørder, M., & Rej, R. (1986). Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. *Journal of Clinical Chemistry and Clinical Biochemistry, 24*, 481-495.

Birketvedt, G. S. (2009). U. S. Patent No. 7,579,027. Washington, DC: U.S. Patent and Trademark Office.

Blanck, H. M., Khan, L. K., & Serdula, M. K. (2001). Use of nonprescription weight loss products. *Journal of the American Medical Association, 286*(8), 930-935.

Blanck, H. M., Serdula, M. K., Gillespie, C., Galuska, D. A., Sharpe, P. A., Conway, J. M., . . . Ainsworth, B. E. (2007). Use of nonprescription dietary supplements for weight loss is common among Americans. *Journal of the American Dietetic Association, 107*(3), 441-447.

Blumenthal, M. (1999). *The complete German commision E monographs, therapeutic guide to herbal medicines: American botanical council*. Austin, TX: Thieme.

Bnouham, M. (2010). Medicinal plants with potential galactagogue activity used in the moroccan pharmacopoeia. *Journal of Complementary and Integrative Medicine, 7*(1), December 2010. doi: 10.2202/1553-3840.1268

Bodeker, G., Ong, C. K., Grundy, C., Burford, G., & Shein, K. (2005). *World Health Organization global atlas of traditional, complementary, and alternative medicine* (Vol. 2). Kobe, Japan: World Health Organization.

Bondiolotti, G., Bareggi, S. R., Frega, N. G., Strabioli, S., & Cornelli, U. (2007). Activity of two different polyglucosamines, L112o and FF45o, on body weight in male rats. *European Journal of Pharmacology, 567*(1-2), 155-158.

Boon, C. S., & Clydesdale, F. M. (2005). A review of childhood and adolescent obesity interventions. *Critical Reviews in Food Science and Nutrition, 45*(7-8), 511-525.

Bown, D. (2001). The herb society of America: New encyclopedia of herbs and their uses (pp. 442). New York: Dorling Kindersley.

Brechbill, G. O. (2012). *The Spice Notes of Fragrance*. New Jersey - USA: Fragrance Books Inc.

Bretaudiere, J., Phung, H., & Bailly, M. (1976). Direct enzymatic determination of urea in plasma and urine with a centrifugal analyzer. *Clinical Chemistry, 22*(10), 1614-1617.

Britton, N. L., & Brown, A. (1913). *An illustrated flora of the northern United States, Canada and the British possessions* (2nd ed. Vol. 2). New York, USA: Charles Scribner's Sons.

Burrin, J., & Price, C. (1985). Measurement of blood glucose. *Annals of Clinical Biochemistry, 22*(4), 327-342.

Butler, A. R. (1975). The Jaffe reaction. Identification of the coloured species. *Clinica Chimica Acta, 59*(2), 227-232.

Caballero, B. (2007). The global epidemic of obesity: An overview. *Epidemiologic Reviews, 29*(1), 1-5.

Calixto, J. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of Medical and Biological Research, 33*(2), 179-189.

Calle, E. E., Rodriguez, C., Walker-Thurmond, K., & Thun, M. J. (2003). Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. *New England Journal of Medicine, 348*(17), 1625-1638.

Can Baser, K. (2008). Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. *Current Pharmaceutical Design, 14*(29), 3106-3119.

Cani, P. D., & Delzenne, N. M. (2011). The gut microbiome as therapeutic target. *Pharmacology & Therapeutics, 130*(2), 202-212. doi: http://dx.doi.org/10.1016/j.pharmthera.2011.01.012

Capasso, F., Gaginella, T. S., Grandolini, G., & Izzo, A. A. (2003). Plants and the digestive system. *Phytotherapy*, 251-294.

Cava, E., & Fontana, L. (2013). Will calorie restriction work in humans? *Aging (Albany NY), 5*(7), 507-514.

Chang, J. (2000). Medicinal herbs: Drugs or dietary supplements? *Biochemical Pharmacology, 59*(3), 211-219.

Chaput, J. P., St-Pierre, S., & Tremblay, A. (2007). Currently available drugs for the treatment of obesity: Sibutramine and orlistat. *Mini Reviews in Medicinal Chemistry, 7*(1), 3-10.

Charles, D. J. (2013). Caraway *Antioxidant properties of spices, herbs and other sources* (pp. 199-206). Norway, IA, USA: Springer

Cheng, T. O. (2006). Obesity is a global challenge. *The American Journal of Medicine, 119*(6), E11-E11.

Chevallier, A. (2000). Encyclopedia of herbal medicine (pp. 336). London: Dorling Kindersley.

Cho, S., Choi, Y., Park, S., & Park, T. (2012). Carvacrol prevents diet-induced obesity by modulating gene expressions involved in adipogenesis and inflammation in mice fed with high-fat diet. *The Journal of Nutritional Biochemistry, 23*(2), 192-201. doi: 10.1016/j.jnutbio.2010.11.016

Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1986). *Glossary of Indian Medicinal Plants (Including the Supplement)*. New Delhi: Council of Scientific & Industrial Research, Publications & Informations Directorate, CSIR.

Clegg, A., Colquitt, J., Sidhu, M., Royle, P., & Walker, A. (2003). Clinical and cost effectiveness of surgery for morbid obesity: A systematic review and economic evaluation. *International Journal of Obesity, 27*(10), 1167-1177.

Clement, K., Viguerie, N., Poitou, C., Carette, C., Pelloux, V., Curat, C. A., . . . Zucker, J.-D. (2004). Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *The FASEB Journal, 18*(14), 1657-1669.

Colman, R. J., Anderson, R. M., Johnson, S. C., Kastman, E. K., Kosmatka, K. J., Beasley, T. M., . . . Weindruch, R. (2009). Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science, 325*(5937), 201-204. doi: 10.1126/science.1173635

Cordell, G. A. (2009). Sustainable drugs and global health care. *Química Nova, 32*(5), 1356-1364.

Cordell, G. A. (2011a). Plant medicines key to global health. *Chemical & Engineering News, 89*(26), 52-56.

Cordell, G. A. (2011b). Sustainable medicines and global health care. *Planta Medica, 77*(11), 1129-1138. doi: 10.1055/s-0030-1270731

Cordell, G. A. (2012). New strategies for traditional medicine. In M. Rai, G. Cordell, J. Martinez, M. Marinoff & L. Rastrelli (Eds.), *Medicinal plants: Biodiversity and drugs* (pp. 1-45). Boca Raton, FL.: CRC Press

Cordell, G. A., & Colvard, M. D. (2012). Natural products and traditional medicine: Turning on a paradigm. *Journal of Natural Products, 75*(3), 514-525.

Craig, C. L., Marshall, A. L., Sjostrom, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., . . . Oja, P. (2003). International physical activity questionnaire: 12-country reliability and validity. *Medicine & Science in Sports & Exercise, 195*(9131/03), 3508-1381.

Dachler, M. (2005). Alimentary, culinary and industrial uses of caraway. In É. Németh (Ed.), *Caraway; the Genus Carum* (pp. 174-185). Amsterdam, The Netherlands: Taylor & Francis e-Library

Dadkhah, A., & Fatemi, F. (2011). Heart and kidney oxidative stress status in septic rats treated with caraway extracts. *Pharmaceutical Biology, 49*(7), 679-686. doi: 10.3109/13880209.2010.539618

Dagenais, G. R., Yi, Q., Mann, J. F., Bosch, J., Pogue, J., & Yusuf, S. (2005). Prognostic impact of body weight and abdominal obesity in women and men with cardiovascular disease. *American Heart Journal, 149*(1), 54-60.

Dallas, C., Gerbi, A., Elbez, Y., Caillard, P., Zamaria, N., & Cloarec, M. (2013). Clinical study to assess the efficacy and safety of a citrus polyphenolic extract of red orange, grapefruit, and orange (Sinetrol-XPur) on weight management and metabolic parameters in healthy overweight individuals. *Phytotherapy Research, 28*(2), 212-218. doi: 10.1002/ptr.4981

Dallas, C., Gerbi, A., Tenca, G., Juchaux, F., & Bernard, F. X. (2008). Lipolytic effect of a polyphenolic citrus dry extract of red orange, grapefruit, orange (SINETROL) in human body fat adipocytes. Mechanism of action by inhibition of cAMP-phosphodiesterase (PDE). *Phytomedicine, 15*(10), 783-792.

Dandona, P., Aljada, A., & Bandyopadhyay, A. (2004). Inflammation: The link between insulin resistance, obesity and diabetes. *Trends in Immunology, 25*(1), 4-7. doi: 10.1016/j.it.2003.10.013

Dandona, P., Aljada, A., Chaudhuri, A., Mohanty, P., & Garg, R. (2005). Metabolic Syndrome A Comprehensive Perspective Based on Interactions Between Obesity, Diabetes, and Inflammation. *Circulation, 111*(11), 1448-1454.

Dara, L., Hewett, J., & Lim, J. K. (2008). Hydroxycut hepatotoxicity: A case series and review of liver toxicity from herbal weight loss supplements. *World Journal of Gastroenterology, 14*(45), 6999-7004.

Das, B. M., & Roy, S. K. (2010). Age changes in the anthropometric and body composition characteristics of the Bishnupriya Manipuris of Cachar district, Assam. *Advances in Bioscience and Biotechnology, 1*(2), 122-130.

Dasgupta, A., & Hammett-Stabler, C. A. (2011). *Herbal supplements: Efficacy, toxicity, interactions with western drugs, and effects on clinical laboratory tests*. Hoboken, New Jersey: John Wiley & Sons, Inc.

Dehghani, F., Panjehshahin, M. R., & Vojdani, Z. (2010). Effect of hydroalcoholic extract of caraway on thyroid gland structure and hormones in female rat. *Iranian Journal of Veterinary research, 11*(4), 337-341.

Delpeuch, F., Maire, B., Monnier, E., & Holdsworth, M. (Eds.). (2013). *Globesity: A planet out of control?* (2nd ed.). London, UK: Routledge.

Delshad, H. (2013). Paper presented at the The 4th National Congress of Obesity, Shahid Beheshti University of Medical Sciences, Tehran, Iran. 4-6 Decemeber 2013.

Discover Life organization. 21/12/2013). *Carum Carvi L. (caraway).* from http://www.discoverlife.org/20/q

Douglas, M., Heyes, J., & Smallfield, B. (2006). Herbs, Spices and Essential Oils: Post-harvest operations in developing countries. *UNIDO/FAO: Vienna, Rome*.

Dwyer, J. T., Allison, D. B., & Coates, P. M. (2005). Dietary supplements in weight reduction. *Journal of the American Dietetic Association, 105*(5), 80-86.

Eddouks, M., Khalidi, A., & Zeggwagh, N. A. (2009). Pharmacological approach of plants traditionally used in treating hypertension in Morocco (Approche pharmacologique des plantes utilisées traditionnellement dans le traitement de l'hypertension artérielle au Maroc). *Phytothérapie, 7*(2), 122-127.

Eddouks, M., Lemhadri, A., & Michel, J. B. (2004). Caraway and caper: Potential anti-hyperglycaemic plants in diabetic rats. *Journal of Ethnopharmacology, 94*(1), 143-148. doi: 10.1016/j.jep

Eddouks, M., Maghrani, M., Lemhadri, A., Ouahidi, M. L., & Jouad, H. (2002). Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). *Journal of Ethnopharmacology, 82*(2-3), 97-103.

El-Shobaki, F., Saleh, Z., & Saleh, N. (1990). The effect of some beverage extracts on intestinal iron absorption. *Zeitschrift für Ernährungswissenschaft, 29*(4), 264-269.

El Amrani, F., Rhallab, A., Alaoui, T., El Badaoui, K., & Chakir, S. (2010). Ethnopharmacological survey of some plants used for the treatment of diabetes in the region of Meknès-Tafilalet (Morocco). *Phytothérapie, 8*(3), 161-165.

Ene, A., Nwankwo, E., & Samdi, L. (2007). Alloxan-induced diabetes in rats and the effects of black caraway (*Carum carvi* L.) oil on their body weight. *Research Journal of Medicine and Medical Sciences, 2*(2), 48-52.

Esmaillzadeh, A., & Azadbakht, L. (2008). Major dietary patterns in relation to general obesity and central adiposity among Iranian women. *The Journal of Nutrition, 138*(2), 358-363.

Esteghamati, A., Khalilzadeh, O., Mohammad, K., Meysamie, A., Rashidi, A., Kamgar, M., . . . Haghazali, M. (2010). Secular trends of obesity in Iran between 1999 and

2007: National surveys of risk factors of non-communicable diseases. *Metabolic Syndrome and Related Disorders, 8*(3), 209-213.

European Information Centre on Complementary and Alternative Medicine - EICCAM. (2013). Retrieved April 2013, from http://www.eiccam.eu/home.php?il=1&l=eng

Fahad, S., & Bano, A. (2012). Ethnobotanical and physiological studies of some endangered plant species collected from two different altitudes in Gilgit Baltistan. *Pakistan Journal of Botany, 44*, 165-170.

Farr, C., & Virchow, K. (2009). Towards a common definition of global health. *The Lancet, 373*, 1993-1995.

Fleiss, J. L. (1986). *The design and analysis of clinical experiments*: Wiley Online Library.

Fogelholm, M., Malmberg, J., Suni, J., Santtila, M., Kyröläinen, H., Mäntysaari, M., & Oja, P. (2006). International Physical Activity Questionnaire: Validity against fitness. *Medicine and Science in Sports and Exercise, 38*(4), 753-760.

Fong, H. H. (2002). Integration of herbal medicine into modern medical practices: Issues and prospects. *Integrative Cancer Therapies, 1*(3), 287-293.

Ford, H., & Carter, J. (1990). Haemostasis in hypothyroidism. *Postgraduate Medical Journal, 66*(774), 280-284.

Fossati, P., & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry, 28*(10), 2077-2080.

Fossati, P., Prencipe, L., & Berti, G. (1980). Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clinical Chemistry, 26*(2), 227-231.

Fouad, M., Rastam, S., Ward, K., & Maziak, W. (2006). Prevalence of obesity and its associated factors in Aleppo, Syria. *Prevention and Control, 2*(2), 85-94.

Furda, I. (1980). U. S. Patent No. 4223023. Washington, DC: U.S. Patent and Trademark Office.

Ghadiri, M. K., & Gorji, A. (2004). Natural remedies for impotence in medieval Persia. *International journal of impotence research, 16*(1), 80-83.

Ghazanfar, S. A. (2011). Medicinal and aromatic plants–Arabia and Iran *Encyclopedia of life support systems*. Oxford, UK: UNESCO-EOLSS.

Gibson, R. S. (2005). *Principles of nutritional assessment*: Oxford university press, USA.

Giovannucci, E., Ascherio, A., Rimm, E. B., Colditz, G. A., Stampfer, M. J., & Willett, W. C. (1995). Physical activity, obesity, and risk for colon cancer and adenoma in men. *Annals of Internal Medicine, 122*, 327-327.

Gledhill, D. (2008). *The names of plants* (4th ed.): Cambridge University Press.

Glenda Winson, S. K. (2001). Management of HIV-associated diarrhea and wasting. *Journal of the Association of Nurses in AIDS Care, 12, Supplement*, 55-62. doi: http://dx.doi.org/10.1016/S1055-3290(06)60158-1

Goodman, D. S., Hulley, S. B., Clark, L. T., Davis, C., Fuster, V., LaRosa, J. C., . . . Brown, W. V. (1988). Report of the National Cholesterol Education Program Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults. *Archives of Internal Medicine, 148*(1), 36.

Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B., & Dawber, T. R. (1977). Predicting coronary heart disease in middle-aged and older persons. *Journal of the American Medical Association, 238*(6), 497-499.

Gorji, A., & Khaleghi Ghadiri, M. (2001). History of epilepsy in Medieval Iranian medicine. *Neuroscience & Biobehavioral Reviews, 25*(5), 455-461.

Graham, I., Atar, D., Borch-Johnsen, K., Boysen, G., Burell, G., Cifkova, R., . . . Gjelsvik, B. (2007). European guidelines on cardiovascular disease prevention in clinical practice: Executive summary. *European Heart Journal, 28*(19), 2375-2414.

Greenberg, R. S., Daniels, S. R., Flanders, W., Eley, J., & Boring, J. R. (2001). *Medical Epidemiology* (3 ed.). London: McGraw-Hill.

Greenway, F., de Jonge-Levitan, L., Martin, C., Roberts, A., Grundy, I., & Parker, C. (2006). Dietary herbal supplements with phenylephrine for weight loss. *Journal of Medicinal Food, 9*(4), 572-578.

Greenway, F., Liu, Z., Martin, C., Kai-Yuan, W., Nofziger, J., Rood, J., . . . Amen, R. (2006). Safety and efficacy of NT, an herbal supplement, in treating human obesity. *International Journal of Obesity, 30*(12), 1737-1741.

Grieve, M. (1971). *A modern herbal: The medicinal, culinary, cosmetic and economic properties, cultivation and folklore of herbs, grasses, fungi, shrubs, & trees with all their modern scientific uses* (Vol. 2). New York: Courier Dover Publications.

Guarrera, P. M., & Savo, V. (2013). Perceived health properties of wild and cultivated food plants in local and popular traditions of Italy: A review. *Journal of Ethnopharmacology, 146*(3), 659-680. doi: http://dx.doi.org/10.1016/j.jep.2013.01.036

Guh, D., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C. L., & Anis, A. (2009). The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. *BMC Public Health, 9*(88), 1-20.

Haaz, S., Fontaine, K., Cutter, G., Limdi, N., Perumean-Chaney, S., & Allison, D. (2006). *Citrus aurantium* and synephrine alkaloids in the treatment of overweight and obesity: An update. *Obesity Reviews, 7*(1), 79-88.

Hagströmer, M., Oja, P., & Sjöström, M. (2006). The International Physical Activity Questionnaire (IPAQ): A study of concurrent and construct validity. *Public Health Nutrition, 9*(06), 755-762.

Haidari, F., Seyed-Sadjadi, N., Taha-Jalali, M., & Mohammed-Shahi, M. (2011). The effect of oral administration of *Carum carvi* on weight, serum glucose, and lipid profile in streptozotocin-induced diabetic rats. *Saudi Medical Journal, 32*(7), 695-700.

Halberstein, R. A. (2005). Medicinal plants: Historical and cross-cultural usage patterns. *Annals of Epidemiology, 15*(9), 686-699. doi: http://dx.doi.org/10.1016/j.annepidem.2005.02.004

Hammer, K., Lehmann, C. O., & L'errino, P. (1988). A check-list of the Libyan cultivated plants including an inventory of the germplasm collected in the years 1981, 1982 and 1983. *Genetic Resources and Crop Evolution, 36*(3), 475-527.

Hansen, D. K., George, N. I., White, G. E., Abdel-Rahman, A., Pellicore, L. S., & Fabricant, D. (2013). Cardiovascular Toxicity of *Citrus aurantium* in Exercised Rats. *Cardiovascular toxicology*, 1-12.

Hasani-Ranjbar, S., Jouyandeh, Z., & Abdollahi, M. (2013). A systematic review of anti-obesity medicinal plants-an update. *Journal of Diabetes & Metabolic Disorders, 12:28*(1).

Haslam, D., Sattar, N., & Lean, M. (2006). ABC of obesity: Obesity-time to wake up. *British Medical Journal, 333*(7569), 640-642.

Hauptman, J., Lucas, C., Boldrin, M. N., Collins, H., & Segal, K. R. (2000). Orlistat in the long-term treatment of obesity in primary care settings. *Archives of Family Medicine, 9*(2), 160.

Hawrelak, J. A., Cattley, T., & Myers, S. P. (2009). Essential oils in the treatment of intestinal dysbiosis: A preliminary *in vitro* study. *Alternative Medicine Review, 14*(4), 380-384.

Heber, D. (2003). Herbal preparations for obesity: Are they useful? *Primary care, 30*(2), 441-463.

Hollander, P. (2003). Orlistat in the treatment of obesity. *Primary care, 30*(2), 427.

Hopkins, J. T., McLoda, T. A., Seegmiller, J. G., & Baxter, G. D. (2004). Low-level laser therapy facilitates superficial wound healing in humans: A triple-blind, sham-controlled study. *Journal of Athletic training, 39*(3), 223.

Hørder, M., Magid, E., Pitkänen, E., Härkönen, M., Strömme, J., Theodorsen, L., . . . Waldenström, J. (1979). Recommended method for the determination of creatine

kinase in blood modified by the inclusion of EDTA: The Committee on Enzymes of The Scandinavian Society for Clinical Chemistry and Clinical Physiology (SCE). *Scandinavian Journal of Clinical & Laboratory Investigation, 39*(1), 1-5.

Howard, N. J., Taylor, A. W., Gill, T. K., & Chittleborough, C. R. (2008). Severe obesity: Investigating the socio-demographics within the extremes of body mass index. *Obesity Research & Clinical Practice, 2*(1), 51-59.

Hsu, C. L., & Yen, G. C. (2007a). Effects of capsaicin on induction of apoptosis and inhibition of adipogenesis in 3T3-L1 cells. *Journal of Agricultural and Food Chemistry, 55*(5), 1730-1736.

Hsu, C. L., & Yen, G. C. (2007b). Effects of flavonoids and phenolic acids on the inhibition of adipogenesis in 3T3-L1 adipocytes. *Journal of Agricultural and Food Chemistry, 55*(21), 8404-8410.

Hsu, C. L., & Yen, G. C. (2008). Phenolic compounds: Evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms. *Molecular Nutrition & Food Research, 52*(1), 53-61. doi: 10.1002/mnfr.200700393

Hsu, T. F., Kusumoto, A., Abe, K., Hosoda, K., Kiso, Y., Wang, M. F., & Yamamoto, S. (2006). Polyphenol-enriched oolong tea increases fecal lipid excretion. *European Journal of Clinical Nutrition, 60*(11), 1330-1336.

Iacobellis, N. S., Lo Cantore, P., Capasso, F., & Senatore, F. (2005). Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *Journal of Agricultural and Food Chemistry, 53*(1), 57-61.

International Obesity Taskforce. (2010). *Obesity the Global Epidemic*. from http://www.iaso.org/iotf/obesity/obesitytheglobalepidemic/

Ishihara, K., Oyaizu, S., Fukuchi, Y., Mizunoya, W., Segawa, K., Takahashi, M., . . . Yasumoto, K. (2003). A soybean peptide isolate diet promotes postprandial carbohydrate oxidation and energy expenditure in type II diabetic mice. *The Journal of Nutrition, 133*(3), 752-757.

Iyer, A., Fairlie, D. P., Prins, J. B., Hammock, B. D., & Brown, L. (2010). Inflammatory lipid mediators in adipocyte function and obesity. *Nature Reviews Endocrinology, 6*(2), 71-82. doi: 10.1038/nrendo.2009.264

Jacobs, B., & Gundling, K. (2009). *ACP evidence-based guide to complementary and alternative medicine*: American College of Physicians.

James, P. T. (2008). The epidemiology of obesity: The size of the problem. *Journal of Internal Medicine, 263*(4), 336-352. doi: 10.1111/j.1365-2796.2008.01922.x

Jiménez-Cruz, A., Manuel Loustaunau-López, V., & Bacardi-Gascón, M. (2006). The use of low glycemic and high satiety index food dishes in Mexico: A low cost approach to prevent and control obesity and diabetes. *Nutrición Hospitalaria, 21*(3), 353-356.

Jin, H., & Jiao, D. (1994). Effect of Jiang-Zhi Jian-Fei Yao on gastro-intestinal movement and adipose cell of abdominal wall. *Chinese Journal of Integrated Traditional and Western Medicine, 14*(4), 230.

Johnstone, A. M., Murison, S. D., Duncan, J. S., Rance, K. A., & Speakman, J. R. (2005). Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *American Journal of Clinical Nutrition, 82*(5), 941-948.

Johri, R. K. (2011). *Cuminum cyminum* and *Carum carvi*: An update. *Pharmacognosy Reviews, 5*(9), 63-72. doi: 10.4103/0973-7847.79101

Jones, F. A. (1996). Herbs: Useful plants. *Journal of the Royal Society of Medicine, 89*(12), 717.

Joshi, S. G. (2000). *Medicinal plants: Family Apiaceae* (1st ed.): Oxford and IBH Publishing Co. Pvt. Ltd.

Jouad, H., Haloui, M., Rhiouani, H., El Hilaly, J., & Eddouks, M. (2001). Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez–Boulemane). *Journal of Ethnopharmacology, 77*(2), 175-182.

Jun, S., Jung, E., Kang, D., Kim, J., Chang, U., & Suh, H. (2010). Vitamin C increases the fecal fat excretion by chitosan in guinea-pigs, thereby reducing body weight gain. *Phytotherapy Research, 24*(8), 1234-1241.

Kalle, R., & Soukand, R. (2012). Historical ethnobotanical review of wild edible plants of Estonia (1770s-1960s). *Acta Societatis Botanicorum Poloniae, 81*(4), 271-281. doi: 10.5586/asbp.2012.033

Kalupahana, N. S., Claycombe, K. J., & Moustaid-Moussa, N. (2011). (n-3) Fatty Acids Alleviate Adipose Tissue Inflammation and Insulin Resistance: Mechanistic Insights. *Advances in Nutrition, 2*(4), 304-316. doi: 10.3945/an.111.000505

Kamaleeswari, M., Deeptha, K., Sengottuvelan, M., & Nalini, N. (2006). Effect of dietary caraway (*Carum carvi* L.) on aberrant crypt foci development, fecal steroids, and intestinal alkaline phosphatase activities in 1, 2-dimethylhydrazine-induced colon carcinogenesis. *Toxicology and Applied Pharmacology, 214*(3), 290-296.

Kang, S. A., Hong, K., Jang, K. H., Kim, Y. Y., Choue, R., & Lim, Y. (2006). Altered mRNA expression of hepatic lipogenic enzyme and PPARα in rats fed dietary levan from '*Zymomonas mobilis*'. *The Journal of Nutritional Biochemistry, 17*(6), 419-426.

Kenner, D., & Requena, Y. (2001). *Botanical medicine: A European professional perspective*. Brookline, Massachusetts 02445 USA: Paradigm Publications.

Khan, I. A., & Abourashed, E. A. (2011). *Leung's encyclopedia of common natural ingredients: Used in food, drugs and cosmetics* (3rd ed.). Hoboken, New Jersey: John Wiley & Sons, Inc.

Khare, C. P. (Ed.) (2008) Indian medicinal plants: An illustrated dictionary. New Delhi, India: Springer.

Kim, B. (2008). Thyroid hormone as a determinant of energy expenditure and the basal metabolic rate. *Thyroid, 18*(2), 141-144. doi: 10.1089/thy.2007.0266

Kim, H. J., Bae, I. Y., Ahn, C. W., Lee, S., & Lee, H. G. (2007). Purification and identification of adipogenesis inhibitory peptide from black soybean protein hydrolysate. *Peptides, 28*(11), 2098-2103.

Kim, J. H., Hahm, D. H., Yang, D. C., Lee, H. J., & Shim, I. (2005). Effect of Crude Saponin of Korean Red Ginseng on High Fat Diet-Induced Obesity in the Rat. *Journal of Pharmacological Sciences, 97*(1), 124-131

Klein, G., Kim, J., Himmeldirk, K., Cao, Y., & Chen, X. (2007). Antidiabetes and anti-obesity activity of *Lagerstroemia speciosa*. *Evidence-Based Complementary and Alternative Medicine, 4*(4), 401-408.

Knechtle, B., Wirth, A., Knechtle, P., Rosemann, T., Rüst, C., & Bescós, R. (2011). A comparison of fat mass and skeletal muscle mass estimation in male ultra-endurance athletes using bioelectrical impedance analysis and different anthropometric methods. *Nutrición Hospitalaria, 26*(6), 1420-1427.

Koo, S. I., & Noh, S. K. (2007). Green tea as inhibitor of the intestinal absorption of lipids: Potential mechanism for its lipid-lowering effect. *The Journal of Nutritional Biochemistry, 18*(3), 179-183.

Kopelman, P. G. (2000). Obesity as a medical problem. *Nature, 404*(6778), 635-643.

Kottke, T. E., Wu, L. A., & Hoffman, R. S. (2003). Economic and psychological implications of the obesity epidemic. *Mayo Clinic Proceedings, 78*(1), 92-94.

Kruger, J., Galuska, D. A., Serdula, M. K., & Jones, D. A. (2004). Attempting to lose weight: Specific practices among US adults. *American Journal of Preventive Medicine, 26*(5), 402-406.

Kumar, D., Kumar, A., & Prakash, O. (2012). Potential antifertility agents from plants: A comprehensive review. *Journal of Ethnopharmacology, 140*(1), 1-32. doi: http://dx.doi.org/10.1016/j.jep.2011.12.039

Kumari, P., Singh, N., Bhatia, V., Chawla, & Kumar, D. (2011). Herbal fight for obesity: A review. *International Journal of Pharmaceutical Research and Development, 3*(4), 25-28.

Kunzemann, J., & Herrmann, K. (1977). Isolation and identification of flavon (ol)-O-glycosides in caraway (*Carum carvi L.*), fennel (*Foeniculum vulgare Mill.*), anise (*Pimpinella anisum* L.), and coriander (*Coriandrum sativum* L.), and of flavon-C-glycosides in anise. I. Phenolics of spices (author's transl)]. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung, 164*(3), 194-200.

Lahlou, S., Tahraoui, A., Israili, Z., & Lyoussi, B. (2007). Diuretic activity of the aqueous extracts of *Carum carvi* and Tanacetum vulgare in normal rats. *Journal of Ethnopharmacology, 110*(3), 458-463. doi: 10.1016/j.jep.2006.10.005

Landin, K., Stigendal, L., Eriksson, E., Krotkiewski, M., Risberg, B., Tengborn, L., & Smith, U. (1990). Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. *Metabolism, 39*(10), 1044-1048.

Laribi, B., Kouki, K., Bettaieb, T., Mougou, A., & Marzouk, B. (2013). Essential oils and fatty acids composition of Tunisian, German and Egyptian caraway (*Carum carvi* L.) seed ecotypes: A comparative study. *Industrial Crops and Products, 41*(0), 312-318. doi: http://dx.doi.org/10.1016/j.indcrop.2012.04.060

Lau, D. C. W., Douketis, J. D., Morrison, K. M., Hramiak, I. M., Sharma, A. M., & Ur, E. (2007). 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary]. *Canadian Medical Association Journal, 176*(8), S1-S13.

Lebovitz, H. E. (2003). The relationship of obesity to the metabolic syndrome. *International Journal of Clinical Practice. Supplement*(134), 18.

Lee, C. M. Y., Huxley, R. R., Wildman, R. P., & Woodward, M. (2008). Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: A meta-analysis. *Journal of Clinical Epidemiology, 61*(7), 646-653.

Lei, F., Zhang, X. N., Wang, W., Xing, D. M., Xie, W. D., Su, H., & Du, L. J. (2007). Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. *International Journal of Obesity, 31*(6), 1023-1029. doi: 10.1038/sj.ijo.0803502

Lemhadri, A., Hajji, L., Michel, J. B., & Eddouks, M. (2006). Cholesterol and triglycerides lowering activities of caraway fruits in normal and streptozotocin diabetic rats. *Journal of Ethnopharmacology, 106*(3), 321-326. doi: 10.1016/j.jep.2006.01.033

Levetin, E., & McMahon, K. (1999). Plants and society, WCB (2nd ed.): McGraw Hill, Boston, Massachusetts, USA.

Lim, T. (2013). *Carum carvi Edible Medicinal And Non-Medicinal Plants* (Vol. 5, Fruits, pp. 6-18): Springer Science+Business Media Dordrecht

Lombardo, Y. B., & Chicco, A. G. (2006). Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. *Journal of Nutritional Biochemistry, 17*(1), 1-13. doi: 10.1016/j.jnutbio.2005.08.002

Maddock, J. (2004). The relationship between obesity and the prevalence of fast food restaurants: State-level analysis. *American Journal of Health Promotion, 19*(2), 137-143.

Madisch, A., Holtmann, G., Plein, K., & Hotz, J. (2004). Treatment of irritable bowel syndrome with herbal preparations: Results of a double-blind, randomized, placebo-controlled, multi-centre trial. *Alimentary Pharmacology & Therapeutics, 19*(3), 271-279.

Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., & Miyashita, K. (2005). Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochemical and Biophysical Research Communications, 332*(2), 392-397.

Maeda, H., Hosokawa, M., Sashima, T., & Miyashita, K. (2007). Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice. *Journal of Agricultural and Food Chemistry, 55*(19), 7701-7706.

Maeda, H., Hosokawa, M., Sashima, T., Takahashi, N., Kawada, T., & Miyashita, K. (2006). Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *International Journal of Molecular Medicine, 18*(1), 147-152.

Maeda, H., Tsukui, T., Sashima, T., Hosokawa, M., & Miyashita, K. (2008). Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. *Asia Pacific Journal of Clinical Nutrition, 17*(S1), 196-199.

Mandal, G. C., Bose, K., & Kozieł, S. (2011). Impact of social class on body fatness among rural pre-school Bengalee Hindu children of Arambagh, West Bengal, India. *Journal of Comparative Human Biology, 62*(3), 228-236. doi: http://dx.doi.org/10.1016/j.jchb.2011.03.001

Mariaca, R. G., Berger, T. F. H., Gauch, R., Imhof, M. I., Jeangros, B., & Bosset, J. O. (1997). Occurrence of volatile mono-and sesquiterpenoids in highland and lowland plant species as possible precursors for flavor compounds in milk and dairy products. *Journal of Agricultural and Food Chemistry, 45*(11), 4423-4434.

Martins, F., Noso, T. M., Porto, V. B., Curiel, A., Gambero, A., Bastos, D. H. M., . . . Carvalho, P. O. (2009). Maté tea inhibits *in vitro* pancreatic lipase activity and has hypolipidemic effect on high-fat diet-induced obese mice. *Obesity, 18*(1), 42-47.

Masango, P. (2005). Cleaner production of essential oils by steam distillation. *Journal of Cleaner Production, 13*(8), 833-839.

Masuo, K., Kawaguchi, H., Mikami, H., Ogihara, T., & Tuck, M. L. (2003). Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. *Hypertension, 42*(4), 474-480.

Matsumura, T., Ishikawa, T., & Kitajima, J. (2001). New p-menthanetriols and their glucosides from the fruit of caraway. *Tetrahedron, 57*(38), 8067-8074.

Matsumura, T., Ishikawa, T., & Kitajima, J. (2002a). Water-soluble constituents of caraway: Aromatic compound, aromatic compound glucoside and glucides. *Phytochemistry, 61*(4), 455-459.

Matsumura, T., Ishikawa, T., & Kitajima, J. (2002b). Water-soluble constituents of caraway: Carvone derivatives and their glucosides. *Chemical and Pharmaceutical Bulletin, 50*(1), 66-72.

Mattes, R. D., & Bormann, L. (2000). Effects of (-)-hydroxycitric acid on appetitive variables. *Physiology & Behavior, 71*(1), 87-94.

Maury, E., & Brichard, S. M. (2010). Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Molecular and Cellular Endocrinology, 314*(1), 1-16. doi: 10.1016/j.mce.2009.07.031

May, B., Kohler, S., & Schneider, B. (2000). Efficacy and tolerability of a fixed combination of peppermint oil and caraway oil in patients suffering from functional dyspepsia. *Alimentary Pharmacology & Therapeutics, 14*(12), 1671-1677. doi: 10.1046/j.1365-2036.2000.00873.x

McCrory, M. A., Hamaker, B. R., Lovejoy, J. C., & Eichelsdoerfer, P. E. (2010). Pulse consumption, satiety, and weight management. *Advances in Nutrition, 1*(1), 17-30.

McKenna, T. K. (1999). *Food of the Gods: The search for the original tree of knowledge: a radical history of plants, drugs, and human evolution*. New York: Random House.

McLeod, A. I. (1985). Remark AS R58: A remark on algorithm AS 183. An efficient and portable pseudo-random number generator. *Journal of the Royal Statistical Society. Series C (Applied Statistics), 34*(2), 198-200.

Meckling, K. A., O'Sullivan, C., & Saari, D. (2004). Comparison of a low-fat diet to a low-carbohydrate diet on weight loss, body composition, and risk factors for diabetes and cardiovascular disease in free-living, overweight men and women. *Journal of Clinical Endocrinology & Metabolism, 89*(6), 2717-2723. doi: 10.1210/jc.2003-031606

Mhaskar, K., Blatter, E., & Caius, J. (2000). *Kiritikar and Basu's Illustrated Indian Medicinal Plants, their usages in Ayurveda and Unani Medicines* (Vol. 5th). New Delhi: Sri Satguru publications.

Michiels, J., Missotten, J., Fremaut, D., De Smet, S., & Dierick, N. (2007). *In vitro* dose-response of carvacrol, thymol, eugenol and *trans*-cinnamaldehyde and interaction of combinations for the antimicrobial activity against the pig gut flora. *Livestock Science, 109*(1-3), 157-160. doi: 10.1016/j.livsci.2007.01.132

Mikaili, P., Shayegh, J., Asghari, M. H., Sarahroodi, S., & Sharifi, M. (2011). Currently used traditional phytomedicines with hot nature in Iran. *Annals of Biological Research, 2*(5), 56-68.

Mirmiran, P., Hosseini Esfahani, F., Mehrabi, Y., Hedayati, M., & Azizi, F. (2010). Reliability and relative validity of an FFQ for nutrients in the Tehran Lipid and Glucose Study. *Public Health Nutrition, 13*(05), 654-662.

Mirzazadeh, A., Sadeghirad, B., Haghdoost, A., Bahreini, F., & Kermani, M. R. (2009). The prevalence of obesity in Iran in recent decade; a systematic review and meta-analysis study. *Iranian Journal of Public Health, 38*(3).

Mittal, R., Goyal, M. M., Dasude, R. C., Quazi, S. Z., & Basak, A. (2011). Measuring obesity: Results are poles apart obtained by BMI and bio-electrical impedance analysis. *Journal of Biomedical Science and Engineering, 4*(11), 677-683.

Mokdad, A. H., Marks, J. S., Stroup, D. F., & Gerberding, J. L. (2004). Actual causes of death in the United States, 2000. *Journal of the American Medical Association, 291*(10), 1238-1245.

Moreno, M., de Lange, P., Lombardi, A., Silvestri, E., Lanni, A., & Goglia, F. (2008). Metabolic effects of thyroid hormone derivatives. *Thyroid, 18*(2), 239-253.

Mosaddegh, M., & Naghibi, F. (Eds.). (2001). *Iran's traditional medicine, past and present* (Vol. 1). Tehran, Iran: Traditional Medicine & Materica Media Research Center.

Moss, D. W., & Henderson, A. R. (2001). Principles of clinical enzymology. *Tietz Fundamentals of Clinical Chemistry. 5th ed. Philadelphia, PA: Saunders*, 157-176.

Muls, E., Kolanowski, J., Scheen, A., & Van Gaal, L. (2001). The effects of orlistat on weight and on serum lipids in obese patients with hypercholesterolemia: A randomized, double-blind, placebo-controlled, multicentre study. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 25*(11), 1713.

Musso, G., Gambino, R., & Cassader, M. (2011). Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. *Annual Review of Medicine, 62*, 361-380. doi: 10.1146/annurev-med-012510-175505

Naderi-Kalali, B., Allameh, A., Rasaee, M. J., Bach, H. J., Behechti, A., Doods, K., . . . Schramm, K. W. (2005). Suppressive effects of caraway (*Carum carvi*) extracts on 2, 3, 7, 8-tetrachloro-dibenzo-*p*-dioxin-dependent gene expression of cytochrome P450 1A1 in the rat H4IIE cells. *Toxicology in Vitro, 19*(3), 373-377.

Nagao, T., Hase, T., & Tokimitsu, I. (2007). A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity, 15*(6), 1473-1483.

Naito, H. (2003). Coronary artery disease and disorders of lipid metabolism. *Clinical Chemistry: Theory, Analysis, Correlation, 4th Ed., Kaplan, LA, Pesce, AJ, Kazmierczak, SC (Mosby, Inc. eds. St Louis USA), 603*.

Najda, A., Dyduch, J., & Brzozowski, N. (2008). Flavonoid content and antioxidant activity of caraway roots (*Carum carvi* L.). *Vegetable Crops Research Bulletin, 68*(1), 127-133.

Nakai, M., Fukui, Y., Asami, S., Toyoda-Ono, Y., Iwashita, T., Shibata, H., . . . Kiso, Y. (2005). Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. *Journal of Agricultural and Food Chemistry, 53*(11), 4593-4598.

Nakano, Y., Matsunaga, H., Saita, T., Mori, M., Katano, M., & Okabe, H. (1998). Antiproliferative constituents in Umbelliferae plants II. Screening for polyacetylenes in some Umbelliferae plants, and isolation of panaxynol and falcarindiol from the root of Heracleum moellendorffii. *Biological & Pharmaceutical Bulletin, 21*(3), 257-261.

Nasser, M., Tibi, A., & Savage-Smith, E. (2009). Ibn Sina's Canon of Medicine: 11th century rules for assessing the effects of drugs. *Journal of the Royal Society of Medicine, 102*(2), 78-80. doi: 10.1258/jrsm.2008.08k040

National Center for Health Statistics NHANES. (2004). *CDC (Centers for Disease Control and Prevention) NHANES 2003–2004 Laboratory Procedures Manual.* Retrieved 3 November, 2008, from http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/lab_pm.pdf

National Institutes of Health. (2000). *The practical guide: Identification, evaluation, and treatment of overweight and obesity in adults*. Maryland: US.

National Medical Advisory Committee; Scottish Office Department of Health; Complementary Medicine and the National Health Services. (1996). An examination of Acupuncture, Homeopathy, Chropractic and Osteopathy.

Natural Resources Conservation Service; United States Department of Agriculture: USDA-NRCS. (2013, 2 December 2013). *Plants Classification Report: Family Apiaceae.* from http://plants.usda.gov/java/ClassificationServlet?source=profile&symbol=Apiaceae&display=31

Nayebi, F. (2011). Association between human body composition and periodontal disease. *ISRN Dentistry, 2011*.

Nestle, M., & Jacobson, M. F. (2000). Halting the obesity epidemic: A public health policy approach. *Public Health Reports, 115*(1), 12-24.

Newman, D., & Price, C. (2001). Nonprotein nitrogen metabolites. *Tietz Fundumentals of Clinical Chemistry. 5thed. Philadelphia, WB Saunders2001*.

Nicotra, G. (2012). Phytotherapy of functional dyspepsia Herbs for gastric discomfort. *Agro Food Industry Hi-Tech, 23*(5), 24-27.

Nikooyeh, B., Neyestani, T. R., Farvid, M., Alavi-Majd, H., Houshiarrad, A., Kalayi, A., . . . Tayebinejad, N. (2011). Daily consumption of vitamin D–or vitamin D+ calcium–fortified yogurt drink improved glycemic control in patients with type 2 diabetes: A randomized clinical trial. *The American Journal of Clinical Nutrition, 93*(4), 764-771.

Nissen, S. L., & Sharp, R. L. (2003). Effect of dietary supplements on lean mass and strength gains with resistance exercise: A meta-analysis. *Journal of Applied Physiology, 94*(2), 651-659.

Ohkoshi, E., Miyazaki, H., Shindo, K., Watanabe, H., Yoshida, A., & Yajima, H. (2007). Constituents from the leaves of *Nelumbo nucifera* stimulate lipolysis in the white adipose tissue of mice. *Planta Medica, 73*(12), 1255-1259.

Okuda, H., Han, L., Kimura, Y., Saito, M., & Murata, T. (2001). Anti-Obesity Action of Herb Tea.(Part 1). Effects or Various Herb Teas on Noradrenaline-Induced Lipolysis in Rat Fat Cells and Pancreatic Lipase Activity. *Japanese Journal of Constitutional Medicine, 63*(1/2), 60-65.

Olshansky, S. J., Passaro, D. J., Hershow, R. C., Layden, J., Carnes, B. A., Brody, J., . . . Ludwig, D. S. (2005). A potential decline in life expectancy in the United States in the 21st century. *New England Journal of Medicine, 352*(11), 1138-1145.

Omodei, D., Licastro, D., Salvatore, F., Crosby, S. D., & Fontana, L. (2013). Serum from humans on long-term calorie restriction enhances stress resistance in cell culture. *Aging (Albany NY), 5*(8), 599-606.

Opala, T., Rzymski, P., Pischel, I., Wilczak, M., & Wozniak, J. (2006). Efficacy of 12 weeks supplementation of a botanical extract-based weight loss formula on body weight, body composition and blood chemistry in healthy, overweight subjects-a randomised double-blind placebo-controlled clinical trial. *European Journal of Medical Research, 11*(8), 343-350.

Padwal, R. S., & Majumdar, S. R. (2007). Drug treatments for obesity: Orlistat, sibutramine, and rimonabant. *The Lancet, 369*(9555), 71-77.

Panton, L. B., Rathmacher, J. A., Baier, S., & Nissen, S. (2000). Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate (HMB) during resistance training. *Nutrition, 16*(9), 734-739. doi: 10.1016/s0899-9007(00)00376-2

Papini, A., Banci, F., & Nardi, E. (2007). Molecular evidence of polyphyletism in the plant genus *Carum* L. (Apiaceae). *Genetics and Molecular Biology, 30*(2), 475-482.

Park, I. K., Kim, J. N., Lee, Y. S., Lee, S. G., Ahn, Y. J., & Shin, S. C. (2008). Toxicity of plant essential oils and their components against Lycoriella ingenua (Diptera: Sciaridae). *Journal of Economic Entomology, 101*(1), 139-144.

Park, T. S. (2013). USA Patent No. Seoul: KR. Original Assignee Industry-Academic Cooperation Foundation, Yonsei University.

Pasman, W. J., Heimerikx, J., Rubingh, C. M., Van Den Berg, R., O'Shea, M., Gambelli, L., . . . Keizer, H. G. (2008). The effect of Korean pine nut oil on *in vitro* CCK release, on appetite sensations and on gut hormones in post-menopausal overweight women. *Lipids Health Dis, 7*(10), 7-10.

Peewãz, S. R. A. (1986). Ibn Sina'S Medical Works. *Indian Journal of History of Science, 21*(4), 297-314.

Perry, L. M., & Metzger, J. (1980). *Medicinal plants of east and southeast Asia: Attributed properties and uses*. Massachusetts and London: MIT press.

Picot, J., Jones, J., Colquitt, J., Gospodarevskaya, E., Loveman, E., Baxter, L., & Clegg, A. (2009). The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: A systematic review and economic evaluation. *Health Technology Assessment, 13*(41), 1-190, 215-357, iii-iv.

Pittler, M. H., & Ernst, E. (2004). Dietary supplements for body-weight reduction: A systematic review. *The American Journal of Clinical Nutrition, 79*(4), 529-536.

Pittler, M. H., & Ernst, E. (2005). Complementary therapies for reducing body weight: A systematic review. *International Journal of Obesity, 29*(9), 1030-1038.

Pittler, M. H., Schmidt, K., & Ernst, E. (2005). Adverse events of herbal food supplements for body weight reduction: Systematic review. *Obesity Reviews, 6*(2), 93-111.

Plant, O. H., & Miller, G. H. (1926). Effects of carminative volatile oils on the muscular activity of the stomach and colon. *The Journal of Pharmacology and Experimental Therapeutics, 27*(2), 149-164.

Popkin, B. M. (2004). The Nutrition Transition: An Overview of World Patterns of Change. *Nutrition reviews, 62*, S140-S143. doi: 10.1111/j.1753-4887.2004.tb00084.x

Popkin, B. M. (2007). The world is fat. *Scientific American, 297*(3), 88-95.

Pouliot, M.-C., Després, J.-P., Lemieux, S., Moorjani, S., Bouchard, C., Tremblay, A., . . . Lupien, P. J. (1994). Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *The American journal of cardiology, 73*(7), 460-468.

Prance, G. S., & Nesbitt, M. (Eds.). (2005). *The Cultural history of plants: Spices*. New York: Routledge.

Preuss, H. G., DiFerdinando, D., Bagchi, M., & Bagchi, D. (2002). *Citrus aurantium* as a thermogenic, weight-reduction replacement for ephedra: An overview. *Journal of Medicine, 33*(1-4), 247-264.

Raal, A., Arak, E., & Orav, A. (2012). The content and composition of the essential oil Found in *Carum carvi* L. commercial fruits obtained from different countries. *Journal of Essential Oil Research, 24*(1), 53-59. doi: 10.1080/10412905.2012.646016

Radzi, C. W. J. W. M., Ying, P. L., Kiat, P. E., Kazemipoor, M., & Jamungi, A. Z. (2013). Sugar Consumption: Case Study on Adolescents' Canned Drinks Intake. *Advanced Science Letters, 19*(10), 2974-2978.

Rashidi, A., Mohammadpour-Ahranjani, B., Vafa, M., & Karandish, M. (2005). Prevalence of obesity in Iran. *Obesity Reviews, 6*(3), 191-192.

Rashidy-Pour, A., Malek, M., Eskandarian, R., & Ghorbani, R. (2009). Obesity in the Iranian population. *Obesity Reviews, 10*(1), 2-6.

Rayalam, S., Della-Fera, M. A., & Baile, C. A. (2008). Phytochemicals and regulation of the adipocyte life cycle. *The Journal of Nutritional Biochemistry, 19*(11), 717-726.

Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Rezaee, M.-B., Jaimand, K., Alinezhad, S., Saberi, R., & Yoshinari, T. (2009). Chemical composition and antiaflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. *Food Control, 20*(11), 1018-1024. doi: 10.1016/j.foodcont.2008.12.007

Reiter, B., Lechner, M., & Lorbeer, E. (1998). The fatty acid profiles–including petroselinic and cis-vaccenic acid–of different Umbelliferae seed oils. *Lipid/Fett, 100*(11), 498-502.

Reynolds, J. (1996). Essential oils and aromatic carminatives *Martindale-The Extra Pharmacopeia* (28th ed., pp. 670-676). London, UK: Royal Pharmaceutical Society

Richter, J., & Schellenberg, I. (2007). Comparison of different extraction methods for the determination of essential oils and related compounds from aromatic plants and optimization of solid-phase microextraction/gas chromatography. *Analytical and Bioanalytical Chemistry, 387*(6), 2207-2217. doi: 10.1007/s00216-006-1045-6

Rifai, N., Bachorik, P. S., & Albers, J. J. (1999). Lipids, lipoproteins and apolipoproteins. *Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: WB Saunders Company*, 809-861.

Ritz, B. W., & Gardner, E. M. (2006). Malnutrition and energy restriction differentially affect viral immunity. *Journal of Nutrition, 136*(5), 1141-1144.

Rivera, L. L., Vilarem, G., Gomez, R. S., Estrada, M. J., & Feijoo, J. A. V. (2010). Water Soluble Fractions of Caraway (*Carum carvi* L.) Essential Oil. *Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas, 9*(6), 495-500.

Roberti di Sarsina, P. (2007). The social demand for a medicine focused on the person: The contribution of CAM to healthcare and healthgenesis. *Evidence-Based Complementary and Alternative Medicine, 4*(Suppl. 1), 45-51. doi: 10.1093/ecam/nem094

Roberts, A. T., Martin, C. K., Liu, Z., Amen, R. J., Woltering, E. A., Rood, J. C., . . . Greenway, F. L. (2007). The safety and efficacy of a dietary herbal supplement and gallic acid for weight loss. *Journal of Medicinal Food, 10*(1), 184-188.

Rosengarten, J. F. (1969). *The Book of Spices*. Wynnewood, Philadelphia: Livingston Publishing Co.

Ross, R., Dagnone, D., Jones, P. J., Smith, H., Paddags, A., Hudson, R., & Janssen, I. (2000). Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in menA randomized, controlled trial. *Annals of Internal Medicine, 133*(2), 92-103.

Rothacker, D., & Waitman, B. (1997). Effectiveness of a *Garcinia cambogia* and natural caffeine combination in weight loss: A double-blind placebo-controlled pilot study. *International Journal of Obesity, 21*(2), 53-57.

Roy, A., & Nallanayagam, M. (2011). Role of herbal medicine in the management of obesity: A review. *Journal of Pharmacy Research, 4*(7).

Rucker, D., Padwal, R., Li, S. K., Curioni, C., & Lau, D. C. W. (2007). Long term pharmacotherapy for obesity and overweight: Updated meta-analysis. *British Medical Journal, 335*(7631), 1194-1199.

Rudolf, M. C. J., Walker, J., & Cole, T. J. (2007). What is the best way to measure waist circumference? *International Journal of Pediatric Obesity, 2*(1), 58-61.

Saad, B., Azaizeh, H., & Said, O. (2008). Arab herbal medicine. In R. R. Watson & V. R. Preedy (Eds.), *Botanical Medicine in Clinical Practice* (pp. 31-39): CAB International

Saad, B., & Said, O. (2011a). *Greco-Arab and Islamic Herbal Medicine: Traditional System, Ethics, Safety, Efficacy, and Regulatory Issues*. Hoboken, New Jersey: John Wiley & Sons, Inc. Publication.

Saad, B., & Said, O. (2011b). Tradition and perspectives of Greco-Arab and Islamic herbal medicine. In A. Dasgupta & C. A. Hammett-Stabler (Eds.), *Herbal Supplements: Efficacy, Toxicity, Interactions with Western Drugs, and Effects on Clinical Laboratory Tests* (pp. 209-253). Hoboken, New Jersey: John Wiley & Sons, Inc. Publication

Sacks, D. B., Bruns, D. E., Goldstein, D. E., Maclaren, N. K., McDonald, J. M., & Parrott, M. (2002). Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clinical Chemistry, 48*(3), 436-472.

Sadiq, S., Nagi, A., Shahzad, M., & Zia, A. (2010). The reno-protective effect of aqueous extract of *Carum carvi* (black zeera) seeds in streptozotocin induced diabetic nephropathy in rodents. *Saudi Journal of Kidney Diseases and Transplantation, 21*(6), 1058-1065.

Sadowska, A., & Obidoska, G. (2003). Pharmacological uses and toxicology of caraway. In É. Németh (Ed.), *Caraway; The Genus Carum* (pp. 186-196). Amesterdam, The Netherlands: Taylor & Francis e-Library

Said, O., Saad, B., Fulder, S., Khalil, K., & Kassis, E. (2011). Weight loss in animals and humans treated with "Weighlevel", a combination of four medicinal plants used in traditional Arabic and Islamic medicine. *Evidence-Based Complementary and Alternative Medicine,* 1-6. doi: 10.1093/ecam/nen067

Salekzamani, S., Neyestani, T. R., Alavi-Majd, H., Houshiarrad, A., Kalayi, A., Shariatzadeh, N., & Gharavi, A. a. (2011). Is vitamin D status a determining factor for metabolic syndrome? A case-control study. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 4*, 205.

Samojlik, I., Lakic, N., Mimica-Dukic, N., Dakovic-Svajcer, K., & Bozin, B. (2010). Antioxidant and hepatoprotective potential of essential oils of coriander (*Coriandrum sativum* L.) and caraway (*Carum carvi* L.) (Apiaceae). *Journal of Agricultural and Food Chemistry, 58*(15), 8848-8853. doi: 10.1021/jf101645n

Sassi, F. (2009). The obesity epidemic: Analysis of past and projected future trends in selected OECD countries: Organisation for Economics Co-operation and Development.

Sastri, B. N. (1956). The Wealth of India: A dictionary of Indian raw materials and industrial products (Vol. 4). New Delhi: Council of Scientific and Industrial Research (CSIR), Publications and Information Directorate.

Schulz, K. F., & Grimes, D. A. (2002). Blinding in randomised trials: Hiding who got what. *The Lancet, 359*(9307), 696-700. doi: http://dx.doi.org/10.1016/S0140-6736(02)07816-9

Seidell, J. C. (2005). Epidemiology of obesity (pp. 3-14.

Seidler-Lozykowska, K., Baranska, M., Baranski, R., & Krol, D. (2010). Raman analysis of caraway (*Carum carvi* L.) single fruits. Evaluation of essential oil content and its composition. *Journal of Agricultural and Food Chemistry, 58*(9), 5271-5275.

Şekerci, Ö. (2007). The Eastern Origin of English Words. *Journal of Language and Linguistic Studies, 3*(1).

Seo, S.-M., Kim, J., Lee, S.-G., Shin, C.-H., Shin, S.-C., & Park, I.-K. (2009). Fumigant antitermitic activity of plant essential oils and components from ajowan (*Trachyspermum ammi*), allspice (*Pimenta dioica*), caraway (*Carum carvi*), dill (*Anethum graveolens*), geranium (*Pelargonium graveolens*), and litsea (*Litsea cubeba*) oils against Japanese termite (*Reticulitermes speratus Kolbe*). *Journal of Agricultural and Food Chemistry, 57*(15), 6596-6602. doi: 10.1021/jf9015416

Shamsuddin, S. (Ed.). (2011). *A Handbook of Traditional and Complementary Medicine Programme in Malaysia*. Kuala Lumpur: Traditional and Complementary Medicine Division, Ministry of Health; Malaysia.

Sharpe, P. A., Blanck, H. M., Williams, J. E., Ainsworth, B. E., & Conway, J. M. (2007). Use of complementary and alternative medicine for weight control in the United States. *The Journal of Alternative and Complementary Medicine, 13*(2), 217-222.

Shehzad, A., Ha, T., Subhan, F., & Lee, Y. (2011). New mechanisms and the anti-inflammatory role of curcumin in obesity and obesity-related metabolic diseases. *European Journal of Nutrition, 50*(3), 151-161. doi: 10.1007/s00394-011-0188-1

Sheng, X., Zhang, Y., Gong, Z., Huang, C., & Zang, Y. Q. (2008). Improved insulin resistance and lipid metabolism by cinnamon extract through activation of peroxisome proliferator-activated receptors. *PPAR Research, 2008*, 1-9. doi: 10.1155/2008/581348

Sher, H., Alyemeni, M. N., & Faridullah. (2010). Cultivation and domestication study of high value medicinal plant species (its economic potential and linkages with commercialization). *African Journal of Agricultural Research, 5*(18), 2462-2470.

Sherwin, J. (2003). Liver function. In L. A. Kaplan & A. J. Pesce (Eds.), *Clinical Chemistry, theory, analysis, and correlation.* (4th ed., pp. 492-499). St Louis USA: Mosby Inc. eds

Simic, A., Rancic, A., Sokovic, M. D., Ristic, M., Grujic-Jovanovic, S., Vukojevic, J., & Marin, P. D. (2008). Essential oil composition of *Cymbopogon winterianus* and *Carum carvi* and their antimicrobial activities. *Pharmaceutical Biology, 46*(6), 437-441. doi: 10.1080/13880200802055917

Siraisi, N. G. (2001). Renaissance readers and Avicenna's organization of medical knowledge *Medicine and the Italian Universities: 1250-1600* (Vol. 12, pp. 204-207). Boston, Koln: Brill

Sivarajan, V., & Balachandran, I. (1994). *Ayurvedic Drugs and their Plant Sources*. New Delhi: Oxford and IBH Publishing.

Skeat, W. W. (1892). *Principles of English etymology: The native element*. Oxford, London: Clarendon press.

Smyth, S., & Heron, A. (2006). Diabetes and obesity: The twin epidemics. *Nature Medicine, 12*(1), 75-80.

Sofi, F., Abbate, R., Gensini, G. F., & Casini, A. (2010). Accruing evidence on benefits of adherence to the Mediterranean diet on health: An updated systematic review and meta-analysis. *The American Journal of Clinical Nutrition, 92*(5), 1189-1196.

Stohs, S. J., Preuss, H. G., & Shara, M. (2011). The Safety of Citrus aurantium (Bitter Orange) and its Primary Protoalkaloid p-Synephrine. *Phytotherapy Research, 25*(10), 1421-1428.

Sui, Y., Zhao, H., Wong, V., Brown, N., Li, X., Kwan, A., . . . Chan, J. (2012). A systematic review on use of Chinese medicine and acupuncture for treatment of obesity. *Obesity Reviews, 13*(5), 409-430.

Suk, S.-H., Sacco, R. L., Boden-Albala, B., Cheun, J. F., Pittman, J. G., Elkind, M. S., & Paik, M. C. (2003). Abdominal obesity and risk of ischemic stroke the Northern manhattan stroke study. *Stroke, 34*(7), 1586-1592.

Tahraoui, A., El-Hilaly, J., Israili, Z. H., & Lyoussi, B. (2007). Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). *Journal of Ethnopharmacology, 110*(1), 105-117. doi: 10.1016/j.jep.2006.09.011

Teo, C. C., Tan, S. N., Yong, J. W. H., Hew, C. S., & Ong, E. S. (2010). Pressurized hot water extraction (PHWE). *Journal of Chromatography A, 1217*(16), 2484-2494.

Thompson, R. J. (2008). Manchester: U.S. Patent No. US20080069906 A1. Bourque and Associates.

Tietz, N. W. (1995). *Clinical guide to laboratory tests* (3rd ed.). Philadelphia, USA: WB Saunders Co.

Tominaga, S., Sugahara, T., Nishimoto, S., Yamawaki, M., Nakashima, Y., Kishida, T., . . . Yamauchi, S. (2009). The effect of secoisolariciresinol on 3T3-L1 adipocytes and the relationship between molecular structure and activity. *Bioscience, Biotechnology, and Biochemistry, 73*(1), 35-39.

Trepanowski, J. F., Canale, R. E., Marshall, K. E., Kabir, M. M., & Bloomer, R. J. (2011). Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: A summary of available findings. *Nutrition Journal, 10*, 107-120. doi: 10.1186/1475-2891-10-107

Trinder, P. (1969a). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry, 6*, 24-27.

Trinder, P. (1969b). In vitro enzymatic colorimetric method for the estimation of glucose in serum/plasma. *Annals of Clinical Biochemistry, 6*, 24.

Udani, J., Hardy, M., & Madsen, D. C. (2004). Blocking carbohydrate absorption and weight loss: A clinical trial using Phase 2™ brand proprietary fractionated white bean extract. *Alternative Medicine Review, 9*(1), 63-69.

Upreti, R. K., Kannan, A., & Pant, A. (2008). Alterations in rat gut bacteria and intestinal epithelial cells following experimental exposure of antimicrobials. *FEMS Immunology & Medical Microbiology, 54*(1), 60-69.

Uto-Kondo, H., Ohmori, R., Kiyose, C., Kishimoto, Y., Saito, H., Igarashi, O., & Kondo, K. (2009). Tocotrienol suppresses adipocyte differentiation and Akt phosphorylation in 3T3-L1 preadipocytes. *The Journal of Nutrition, 139*(1), 51-57.

Van Heerden, F. R. (2008). *Hoodia gordonii*: A natural appetite suppressant. *Journal of Ethnopharmacology, 119*(3), 434-437.

Vasiliades, J. (1976). Reaction of alkaline sodium picrate with creatinine: I. Kinetics and mechanism of formation of the mono-creatinine picric acid complex. *Clinical Chemistry, 22*(10), 1664-1671.

Vassault, A., Grafmeyer, D., Naudin, C., Dumont, G., Bailly, M., Henny, J., . . . Georges, P. (1986). Protocole de validation de techniques. *Annales de Biologie Clinique, 44*, 686-745.

Vaughan, J., & Geissler, C. (2009). *The new Oxford book of food plants*. London, UK: Oxford University Press.

Vermaak, I., Viljoen, A. M., & Hamman, J. H. (2011). Natural products in anti-obesity therapy. *Natural Product Reports, 28*(9), 1493-1533.

Viner, R. M., Hsia, Y., Neubert, A., & Wong, I. C. K. (2009). Rise in antiobesity drug prescribing for children and adolescents in the UK: A population-based study. *British Journal of Clinical Pharmacology, 68*(6), 844-851.

Wadden, T. A., Berkowitz, R. I., Womble, L. G., Sarwer, D. B., Arnold, M. E., & Steinberg, C. M. (2012). Effects of Sibutramine Plus Orlistat in Obese Women Following 1 Year of Treatment by Sibutramine Alone: A Placebo-Controlled Trial. *Obesity Research, 8*(6), 431-437.

Walsh, D. E., Yaghoubian, V., & Behforooz, A. (1984). Effect of glucomannan on obese patients: A clinical study. *International Journal of Obesity, 8*(4), 289-293.

Wang, Y., Rimm, E. B., Stampfer, M. J., Willett, W. C., & Hu, F. B. (2005). Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *The American Journal of Clinical Nutrition, 81*(3), 555-563.

Wang, Y. C., McPherson, K., Marsh, T., Gortmaker, S. L., & Brown, M. (2011). Health and economic burden of the projected obesity trends in the USA and the UK. *The Lancet, 378*(9793), 815-825.

Weatherley-Jones, E., Nicholl, J. P., Thomas, K. J., Parry, G. J., McKendrick, M. W., Green, S. T., . . . Lynch, S. P. (2004). A randomised, controlled, triple-blind trial of the efficacy of homeopathic treatment for chronic fatigue syndrome. *Journal of Psychosomatic Research, 56*(2), 189-197.

Weigle, D. S. (2003). Pharmacological therapy of obesity: Past, present, and future. *Journal of Clinical Endocrinology & Metabolism, 88*(6), 2462-2469.

Weiss, E. C., Galuska, D. A., Khan, L. K., & Serdula, M. K. (2006). Weight-control practices among US adults, 2001-2002. *American Journal of Preventive Medicine, 31*(1), 18-24.

Wesseltoft-Rao, N., Holven, K. B., Telle-Hansen, V. H., Narverud, I., Iversen, P. O., Hjermstad, M. J., . . . Bye, A. (2012). Measurements of body fat is associated with markers of inflammation, insulin resistance and lipid levels in both overweight and in lean, healthy subjects. *European Society for Clinical Nutrition and Metabolism, 7*(6), e234-e240. doi: http://dx.doi.org/10.1016/j.clnme.2012.10.002

Westphal, J., Horning, M., & Leonhardt, K. (1996). Phytotherapy in functional upper abdominal complaints - Results of a clinical study with a preparation of several plants. *Phytomedicine, 2*(4), 285-291.

Wheatley, D. (2004). Triple-blind, placebo-controlled trial of Ginkgo biloba in sexual dysfunction due to antidepressant drugs. *Human Psychopharmacology: Clinical and Experimental, 19*(8), 545-548.

Wichmann, B. A., & Hill, I. D. (1982). Algorithm AS 183: An efficient and portable pseudo-random number generator. *Journal of the Royal Statistical Society. Series C (Applied Statistics), 31*(2), 188-190.

wikipedia.unicefuganda.org. (2013). *Histroy of medicine.* from http://wikipedia.unicefuganda.org/latest/A/History%20of%20medicine.html

Wilson, P. W., D'Agostino, R. B., Levy, D., Belanger, A. M., Silbershatz, H., & Kannel, W. B. (1998). Prediction of coronary heart disease using risk factor categories. *Circulation, 97*(18), 1837-1847.

Wolf, A. M., & Colditz, G. A. (1998). Current estimates of the economic cost of obesity in the United States. *Obesity Research, 6*(2), 97-106.

Woodgate, D. E., & Conquer, J. A. (2003). Effects of a stimulant-free dietary supplement on body weight and fat loss in obese adults: A six-week exploratory study. *Current Therapeutic Research, 64*(4), 248-262.

World Health Organization. (1998a). *Basic tests for drugs: Pharmaceutical substances, medicinal plant materials and dosage forms*. Geneva: WHO.

World Health Organization. (1998b). Guidelines for the appropriate use of herbal medicines. Manila: WHO Regional Office for the Western Pacific.

World Health Organization. (1998c). *Quality control methods for medicinal plant materials.* Geneva: WHO.

World Health Organization. (2000a). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva: WHO.

World Health Organization. (2000b). *Obesity: preventing and managing the global epidemic,* Report of a WHO Consultation (WHO Technical Report Series 894) (pp. i-xii 1-252). Geneva: WHO.

World Health Organization. (2001). Legal status of traditional medicine and complementary-alternative medicine: a worldwide review. Geneva: WHO.

World Health Organization. (2002). WHO traditional medicine strategy 2002-2005 (WHO/EDM/TRM/2002.1) (pp. 74). Geneva: WHO.

World Health Organization. (2003). Diet, nutrition and the prevention of chronic diseases. *WHO Technical Report Series No. 916*(i-viii), 1-149.

World Health Organization. (2004). WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. Geneva: WHO.

World Health Organization. (2005). National policy on traditional medicine and regulation of herbal medicines: Report of a WHO global survey. Geneva: WHO.

World Health Organization. (2009a). Report of WHO interregional workshop on the use of traditional medicines in primary health care. Geneva: WHO.

World Health Organization. (2009b). Traditional medicine. In: Sixty-second World Health Assembly, 18-22 May 2009. Resolutions and decisions, annexes. Geneva: WHO.

World Health Organization. (2010). Safety issues in the preparation of homoeopathic medicines. Geneva: WHO.

World Health Organization. (2011a). Obesity and overweight, Fact Sheet No. 311. Geneva: WHO Media Centre.

World Health Organization. (2011b). Quality control methods for herbal materials (updated edition of 1998 publication). Geneva: WHO.

World Health Organization. (2012). The regional strategy for traditional medicine in the western pacific (2011-2020) (pp. 60). Manila, Republic of the Philippines: WHO, Regional Office for the Western Pacific.

World Health Organization. (2014a). *Health Topics: Obesity.* from http://www.who.int/topics/obesity/en/

World Health Organization. (2014b). WHO traditional medicine strategy 2014-2023. Geneva: WHO.

Wyatt, S. B., Winters, K. P., & Dubbert, P. M. (2006). Overweight and obesity: Prevalence, consequences, and causes of a growing public health problem. *American Journal of the Medical Sciences, 331*(4), 166-174. doi: 10.1097/00000441-200604000-00002

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., . . . Tartaglia, L. A. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation, 112*(12), 1821-1830.

Yach, D., Stuckler, D., & Brownell, K. D. (2006). Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nature Medicine, 12*(1), 62-66.

Yang, W.-S., Lee, W.-J., Funahashi, T., Tanaka, S., Matsuzawa, Y., Chao, C.-L., . . . Chuang, L.-M. (2001). Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *Journal of Clinical Endocrinology & Metabolism, 86*(8), 3815-3819.

Yoon, W.-J., Lee, N. H., & Hyun, C.-G. (2010). Limonene suppresses lipopolysaccharide-induced production of nitric oxide, prostaglandin E2, and pro-inflammatory cytokines in RAW 264.7 macrophages. *Journal of oleo science, 59*(8), 415-421.

Yun, J. W. (2010). Possible anti-obesity therapeutics from nature – A review. *Phytochemistry, 71*(14–15), 1625-1641. doi: 10.1016/j.phytochem.2010.07.011

Zaid, H., Rayan, A., Said, O., & Saad, B. (2010). Cancer treatment by Greco-Arab and Islamic herbal medicine. *Open Nutraceuticals Journal, 3*, 203-213.

Zargari, A. (1995). *Medicinal plants* (5th ed. Vol. 1). Tehran: Tehran University Publications.

Zhang, C., Rexrode, K. M., van Dam, R. M., Li, T. Y., & Hu, F. B. (2008). Abdominal obesity and the risk of all-cause, cardiovascular, and cancer mortality sixteen years of follow-up in US women. *Circulation, 117*(13), 1658-1667.

List of Publications and Papers Presented

ACADEMIC JOURNALS

Mahnaz Kazemipoor, Che Wan Jasimah Wan Mohamed Radzi, Majid Hajifaraji, Geoffrey A Cordell. “Preliminary safety evaluation and biochemical efficacy of Carum carvi: results from a randomized, triple-blind, and placebo-controlled clinical trial.” Phytotherapy research, 2014, doi:10.1002/ptr.5147 (ISI Thomson Reuters Indexed).

Mahnaz Kazemipoor, Che Wan Jasimah Wan Mohamed Radzi, Majid Hajifaraji, Batoul Sadat Haerian, Mohammad Hossein Mosaddegh, Geoffrey A Cordell. "Antiobesity effect of caraway extract on overweight and obese women: a randomized, triple-blind, placebo-controlled clinical trial," Evidence-Based Complementary and Alternative Medicine, 2013, Article ID 928582, 8 pages, doi: 10.1155/2013/928582.s. (ISI Thomson Reuters Indexed).

Mahnaz Kazemipoor, Geoffrey A. Cordell, Md. Moklesur Rahman Sarker, Che wan Jasimah Bt Wan Mohamed Radzi, Majid Hajifaraji, Phua En Kiat. “Alternative treatments for weight loss: Safety/risks and effectiveness of anti-obesity medicinal plants.” International Journal of Food Properties, 2014 (ISI Thomson Reuters Indexed).

Mahnaz Kazemipoor, Majid Hajifaraji, Che wan Jasimah Bt wan Mohamed Radzi, Shahaboddin Shamshirband, Dalibor Petković. “Appraisal of adaptive neuro-fuzzy computing technique for estimating anti-obesity properties of a medicinal plant.”. Computer Methods and Programs in Biomedicine, 2014, doi: 10.1016/j.cmpb.2014.10.006 (ISI Thomson Reuters Indexed).

Mahnaz Kazemipoor, Che Wan Jasimah Wan Mohamed Radzi, Geoffrey A. Cordell, Iman Yaze. “Safety, Efficacy and Metabolism of Traditional Medicinal Plants in the Management of Obesity: A Review”. International Journal of Chemical Engineering and Applications (IJCEA) (ISSN: 2012-0221), vol. 3, No. 4, pp 288-292 (2012), DOI: 10.7763/IJCEA.2012. V3.201 (Indexed by: Ulrich's Periodicals Directory, Google Scholar, Engineering & Technology Digital Library, and Crossref)

PROCEEDINGS

Mahnaz Kazemipoor, Che Wan Jasimah Wan Mohamed Radzi, Geoffrey A Cordell, Iman Yaze. Potential of Traditional Medicinal Plants for Treating Obesity: A Review, International Conference on Nutrition & Food Science - (ICNFS 2012), July 23-24th, 2012, Singapore (ISI Proceedings). International Proceeding of Chemical, Biological & Environmental Engineering. (ISSN: 2010-4618), vol. 39, pp 164-169 (2012), IACSIT Press, Singapore. Indexed in the Engineering & Technology Digital Library, and indexed by Ei Geobase (Elsevier), Ulrich's Periodicals Directory, EBSCO, CNKI (中国知网), WorldCat, Google Scholar.

INTELLECTUAL PROPERTY RIGHTS (Patent)

“A Herbal composition and method for weight and fat loss management: Potential Anti-Obesity Activity of Caraway Extract Formulated as Dietary Herbal Drink”, Patent, application number: PI 2014700948; UM.TNC2/UMCIC/603/477, Under review, 2014, (Malaysia patent).

The first page of the publications are also appended as reference (Appendix G).

APPENDIX

APPENDIX A: Other aromatic plants mistaken for *carum carvi* due to their resemblance in name or appearance

Appearance	Scientific Name	Common Name	Other names
	*Carum carvi* L.	Caraway seeds/ Persian Cumin	Jiraa, zeera siyaah, kamoon, kamoon-roomi
	Cumin cuminum L.	Cumin	Safed jeeraa, Kamun
	Foeniculum vulgare Mill.	Fennel Seeds/ Sweet Cumin	Perum Jeerakam
	*Pimpinella anisum* L.	Aniseed, anise	Saumph, Aneesun
	Nigella sativa L.	Black Cumin, Black Caraway, small fennel	Kalonji, black seeds
	*Bunium persicum* (Boiss.) B.Fedtsch.	Black Caraway	Jirak, jiraa siyah, Kamoon-armani, Shahi Jeera, Kaala Jeera

Sources: Modified from several sources (Khare, 2008; Zaid *et al.*, 2010)

APPENDIX B: Etymology of caraway (*Carum carvi*)

There is little, incomplete, and yet complex information known about the etymology of *Carum carvi*. The *carvi* term is initiated from the Arabic *al-karwiya* seeds, whereas the word *carum* most likely originated from the Latin word *Caria,* an ancient city in Asia Minor, where caraway may have been used in early times. The usage of "*Carum carvi*" probably dates back to very ancient times based on the finding of caraway seeds by archaeologists in the artefacts of primitive civilizations in Europe (Gledhill, 2008; Şekerci, 2007). In Germany, caraway is one of the most common seeds, and is known as Kümmel. This word is derived from Latin word *cuminum* for cumin, and was used for caraway by mistake. Latin *cuminum*, through Greek kyminon, dates back to Semitic forms, such as Old Hebrew kammon. Some terms for caraway in the languages of Europe, especially Northern Europe (where caraway is predominantly popular), similarly link to Latin *cuminum,* including Danish kommen, Latvian ķimenes, Estonian köömen, Polish kminek, and Bulgarian kim.

The English use of the word caraway was initiated over 1400 years ago. The original term is *cuminum* (cumin) in Latin, and karon in the Greek, which is transformed to carum (which now means caraway). "Karavi", the Sanskrit designation of caraway, and the Arabic term al-karawya is derived from the Latin *carum*. These terms are also similar to some European designations of caraway such as carvi in French, caro in Italian, karvi in Greek, and karve in Norwegian. The English name caraway is most likely mediated by Arabic (modern form al-karawya) from the Latin *carum*. The Iberic terms are in Portuguese, alcaravia, and in Spanish, alcaravea. The English Malayalam terms of caraway are Sheema Jeerakam (Shia Jeera) in Hindi– Caraway seeds/ Persian Cumin/

Meridian Fennel. Some synonyms for caraway in different parts of the world are indicated in APPENDIX B1.

APPENDIX B1: Synonyms of caraway in different languages

Language	Alternative expression
English	Caraway seeds, Persian cumin, Carum, Meridian fennel, Wild cumin
Persian (Farsi)	Black zeereh, Kermani zireh, Roman cumin
Arabic	Taghde, Roman kommon, Al-Karvia, Kammun Armani, Karawiya
Azeri	Adi Cirə
Albanian	Qimnoni
Armenian	Chaman
Finnish	Kumina, Saksan Kumina, Tavallinen, Kumina
Dutch	Karwij, Karwijzaad, Wilde komijn
Chinese	Fang Feng, Ge Lü Zi, Yuan Sui (Mandarin), Goht Leuih Ji (Cantonese)
Bulgarian	Kim
Croatian	Kim
Eastonian	Harilik Köömen
Russian	Тмин, Tmin
Burmese	Ziya
Belarusian	Kmen
Catalan	Comi De Prat
Basque	Xarpoil
Yiddish	Kiml, kimmel
Norwegian	Karve
Czech	Kmín, Kmín Kořenný, Kmín Luční
Dutch	Echte Karwij, Karwij, Karwijzaad, Kummel, Wilde Komijn
Brazil	Alcarávia (Portuguese)
Spanish	Alcarahueya, Alcarave, Alcaravia, Carvi, Comino de prado
French	Anis des Vosges, Carvi, Cumin des Prés, Cumin De Montagne, Kummel
Swedish	Kummin
Gaelic	Carbhaidh, Carvie, Cearbhas, Lus Dearg
German	Gemeiner Kümmel, Wiesenkümmel
Italian	Caro, Carvi, Cumino dei prati, Kümmel, cumino tedesco, German cumin
Danish	Almindelig Kommen, Karve, Kommen, Vild Kommen
Turkish	Frenk kimyonu, Frankish cumin
Hindi	Vilayati jeera, foreign cumin
Galician	Alcaravea, Alcaravía
Romanian	Chimen

Source: Taken/ modified from different sources (Brechbill, 2012; Skeat, 1892)

APPENDIX C: Randomization of candidates through the online randomization program

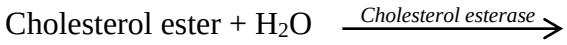
Code No. of respondents	Group type	Code No. of respondents	Group type
1.	Control	36.	Control
2.	Control	37.	Test
3.	Control	38.	Test
4.	Control	39.	Control
5.	Test	40.	Test
6.	Test	41.	Control
7.	Control	42.	Control
8.	Control	43.	Test
9.	Test	44.	Test
10.	Test	45.	Control
11.	Control	46.	Test
12.	Test	47.	Control
13.	Control	48.	Test
14.	Test	49.	Control
15.	Test	50.	Test
16.	Control	51.	Control
17.	Test	52.	Control
18.	Test	53.	Control
19.	Control	54.	Control
20.	Test	55.	Test
21.	Control	56.	Control
22.	Test	57.	Control
23.	Test	58.	Test
24.	Test	59.	Control
25.	Control	60.	Test
26.	Control	61.	Test
27.	Control	62.	Test
28.	Control	63.	Control
29.	Control	64.	Test
30.	Control	65.	Control
31.	Test	66.	Control
32.	Test	67.	Test
33.	Test	68.	Test
34.	Test	69.	Test
35.	Test	70.	Control

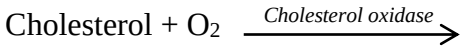
APPENDIX D: Blood test methods

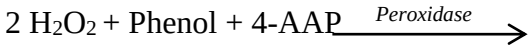
Determination of total serum Cholesterol

Principle:

Enzymatic determination of total cholesterol was according to the following reactions (Allain *et al.*, 1974):







4-AAP = Amino-4-antipyrine

Reagents composition:

Reagent: R			
Pipes buffer, pH 6.7		50	mmol/L
Phenol		24	mmol/L
Sodium cholate		5	mmol/L
4-aminoantipyrine		0.5	mmol/L
Cholesterol esterase	≥	180	U/L
Cholesterol oxidase	≥	200	U/L
Peroxidase	≥	1000	U/L

Reference values:

The NCEP (American National Cholesterol Education Program) has established the following classification for serum total cholesterol levels according to the risk of developing coronary heart diseases (Goodman *et al.*, 1988; Wilson *et al.*, 1998):

Normal	< 200	mg/dl
Borderline high	200-239	mg/dl
High	≥ 240	mg/dl

Procedure:

	Blank	Standard	Sample
Reagent R	300 µL	300 µL	300 µL
Distilled water	3 µL		
Standard		3 µL	
Sample			3 µL

Mixed and the absorbance (A) was read at 500 nm after a 325 seconds incubation at 37 °C. The samples were read against reagent blank.

Calculation:

$(A_{sample} / A_{standard}) \times n$ n = standard concentration

The performance data including analytical range, detection limit, sensitivity, precision and correlation were obtained using the COBAS MIRA analyzer (37 °C).

Determination of serum Cholesterol LDL

Principle:

In first step, when a sample is mixed with reagent R1, non-LDL lipoproteins are solubilized by detergent 1 and released cholesterol is subject to enzymatic reactions to be eliminated:

Cholesterol, HDL, VLDL, Chylomicron $\xrightarrow{Detergent1+CO+CHE}$ Colorless products

In second step, when reagent R2 is added, LDL is solubilized by detergent 2, then LDL cholesterol is measured by enzymatic reactions:

LDL $\xrightarrow{Detergent2}$ Solubilized LDL

LDL cholesterol + O_2 $\xrightarrow{CHE\ +CO}$ Cholest-4-en-3-one + H_2O_2

H_2O_2 + 4-AA + DSBmT $\xrightarrow{Peroxidase}$ Colored compound

Reagents composition:

Reagent1: R1					
MES buffer, pH 6.3					
Detergent 1	<	1.0	%		
Cholesterol esterase (CHE)	<	1500	U/L		
Cholesterol oxidase (CO)	<	1500	U/L		
Peroxidase	<	1300	U/L		
4-Amino-Antipyrine (4-AA)	<	0.1	%		
Ascorbate oxidase	<	3000	U/L		
Reagent2: R2					
MES buffer, pH 6.3					
Detergent 2			<	1.0	%
N,N-bis(4-sulphobutyl)-m-toluidine-disodium (DSBmT)			<	1.0	mmol/L

Reference values:

The NCEP (American National Cholesterol Education Program) has established the following classification for serum LDL cholesterol levels according to the risk of developing coronary heart diseases (Adult Treatment Panel III, 2001):

Optimal	< 100	mg/dl
Near or above optimal	100-129	mg/dl
Borderline high	130-159	mg/dl
High	160-189	mg/dl
Very high	> 190	mg/dl

Procedure:

	Blank	Standard	Sample
Reagent1 R1	240 µL	240 µL	240 µL
Distilled water	2.4 µL	-	-
Standard	-	2.4 µL	-
Sample	-	-	2.4 µL

Mixed and the absorbance (A1) was read at 578 nm after 4 minutes; 40 seconds incubation at 37 °C. Then, reagent 2 (R2) was added.

Reagent2 R2	80 µL

Mixed and after 4 minutes incubation, the absorbance (A2) was measured. The samples were read against reagent blank.

Calculation:

$(A2\text{-}A1)_{sample} / (A2\text{-}A1)_{calibrator} \times n$ n = calibrator concentration

Determination of serum Cholesterol HDL-C

Principle:

Anti-human β-lipoprotein antibody (a mixture containing 5-chloro-2-methyl-2H-isotiazo-3-one [EG No 247-500-7] and 2-methyl-2H-isothiazol-3-one [EG No 220-239-6] (3:1) in R1 binds to lipoproteins (LDL, VLDL and chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reactions when R2 is added. Cholesterol esterase (CHE) and cholesterol oxidase (CO) react only with HDL-C. Hydrogen peroxide produced by the enzyme reactions with HDL-C yields a blue color complex upon oxidative condensation of F-DAOS [N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt] and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD). By measuring the absorbance of the blue color complex produced, the HDL-C concentration in the sample can be calculated when compared with the absorbance of the HDL-C calibrator.

LDL, VLDL and chylomicrons $\xrightarrow{\textit{Anti-human β-lipoprotein antibody}}$ antigen-antibody complex

HDL-Cholesterol + H_2O + O_2 $\xrightarrow{CHE\ \&\ CO}$ Δ^4-Cholesterol + Fatty acid + H_2O_2

Reagents composition:

Reagent1: R1(pretreatment)		
Good's Buffer, pH 7.0	30	mmol/L
4-AA	0.9	mmol/L
POD	2.4	IU/mL
Ascorbate oxidase	2.7	IU/mL
Anti-human β-lipoprotein antibody	0.0015-0.06	%
Reagent2: R2 (Enzyme reagent)		
Good's Buffer, pH 7.0	30	mmol/L
CHE	4.0	IU/mL
CO	20	IU/mL
F-DAOS	0.8	mmol/L

Reference values:

The NCEP (American National Cholesterol Education Program) has established the following classification for serum LDL cholesterol levels according to the risk of developing coronary heart diseases (Adult Treatment Panel III, 2001):

High HDL-cholesterol	> 60	mg/dl
Low HDL-cholesterol	< 40	mg/dl

Procedure:

	Blank	Standard	Sample
Reagent1 R1	270 µL	270 µL	270 µL
Distilled water	2.7 µL	-	-
Standard	-	2.7 µL	-
Sample	-	-	3 µL

Mixed and the absorbance (A1) was read at 600 nm. After 5 minutes incubation at 37 °C the reagent 2 (R2) was added.

Reagent2 R2	90 µL

Mixed and after 5 minutes incubation, the absorbance (A2) was measured. The samples were read against reagent blank.

Calculation:

The final results are automatically calculated and printed in concentration. The results are given in mg/dl.

Determination of Triglycerides

Principle:

Enzymatic determination of triglyceride was according to the following reactions (Fossati & Prencipe, 1982):

$$\text{Triglyceride} + H_2O \xrightarrow{LPL} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{GPO} \text{Dihydroxyacetone-P} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \textit{p}\text{-Chlorophenol} \xrightarrow{Peroxidase} \text{Quinoneimine}$$

4-AAP = Amino-4-antipyrine

LPL = Lipoprotein Lipase

GPO = Glycerol-3-Phosphate oxidase

The samples were serum and heparin or EDTA plasma from fasting patients.

Reagents composition:

Reagent: R			
Pipes buffer, pH 7.0		50	mmol/L
Mg^{2+}		14.8	mmol/L
p-Chlorophenol		2.7	mmol/L
ATP		3.15	mmol/L
Potassium ferrocyanide		10	µmol/L
4-aminoantipyrine		0.31	mmol/L
Lipoprotein lipase	≥	2 000	U/L
Glycerol kinase	≥	500	U/L
Glycerol-3-Phosphate oxidase	≥	4 000	U/L
Peroxidase	≥	500	U/L

Reference values:

The NCEP (American National Cholesterol Education Program) has established the following classification for serum triglyceride levels according to the risk of developing coronary heart diseases:

Normal	< 150	mg/dl
Borderline high	150-200	mg/dl
High	200-500	mg/dl
Very high	≥ 500	mg/dl

Procedure:

	Blank	Standard	Sample
Reagent R	300 µL	300 µL	300 µL
Distilled water	3 µL		
Standard		3 µL	
Sample			3 µL

Mixed and the absorbance (A) was read at 500 nm after a 425 seconds incubation at 37 °C. The samples were read against reagent blank.

Calculation:

$(A_{sample} / A_{standard}) \times n$ n = standard concentration

The performance data including analytical range, detection limit, sensitivity, precision and correlation were obtained using the COBAS MIRA analyzer (37 °C).

Determination of serum urea

Principle:

Enzymatic determination of urea was done (Bretaudiere *et al.*, 1976) according to the following reactions:

$$\text{Urea} + 2\ H_2O \xrightarrow{Urease} 2\ NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \alpha\text{-Ketoglutarate} + NADH \xrightarrow{GIDH} \text{L-Glu} + NAD^+ + H_2O$$

L-Glu = L-Glutamate

GIDH = Glutamate dehydrogenase

The Samples were serum or heparinized plasma.

Reagents composition:

Reagent 1: R1			
Tris buffer, pH 7.6 (37°C)		125	mmol/L
ADP		1	mmol/L
α-Ketoglutarate		9	mmol/L
Urease	≥	8 100	U/L
GIDH	≥	1 350	U/L
Reagent 2: R2			
NADH		1.5	mmol/L
Standard: Std			
Urea		50	mg/dL
		0.5	g/L
		8.32	mmol/L

This standard is included in kits URSL-0407/0427/0507.

Reference values were 10-50 mg/dL in serum, plasma sample.

Procedure:

	Blank	Standard	Sample
Reagent R1	200 µL	200 µL	200 µL
Reagent R2	50 µL	50 µL	50 µL
Distilled water	2.5 µL		
Standard		2.5 µL	
Sample			2.5 µL

Mixed and the variation of absorbance (ΔA) was read at 340 nm between 25 seconds and 75 seconds incubation at 37 °C. The samples were read against reagent blank.

Calculation:

$(\Delta A_{sample} / \Delta A_{standard}) \times n$ n = standard concentration

Determination of serum Creatinine

Principle:

The rate of formation of a coloured complex between Creatinine and alkaline picrate is measured. The effects of interfering substances were reduced using the kinetic procedure. The samples were serum, fluoride or heparinized plasma (Butler, 1975; Vasiliades, 1976).

Reagents composition:

Reagent 1: R1		
Picric acid	8.73	mmol/L
Reagent 2: R2		
Sodium hydorxide	312.5	mmol/L
Disodium phosphate	12.5	mmol/L
Standard: Std		
Creatinine	2	mg/dL

Reference values were 0.4-1.5 mg/dL (for women) in serum, plasma sample.

Procedure:

	Blank	Standard	Sample
Reagent R1	100 µL	200 µL	200 µL
Reagent R2	100 µL	50 µL	50 µL
Distilled water	2.5 µL		
Standard or Sample		20 µL	20 µL

Mixed and the absorbance (A1) was measured at 500 nm after the sample or standard was added at 37 °C incubation. Exactly 2 minutes after reading, the second reading was taken.

Calculation:

$[(A2\text{-}A1)_{sample} / (A2\text{-}A1)_{standard}] \times n$ n = standard concentration

Determination of uric acid

Principle:

Enzymatic determination of uric acid was done (Fossati *et al.*, 1980) according to the following reactions:

$$\text{Uric acid} + 2\ H_2O + O_2 \xrightarrow{Uricase} \text{Allantoin} + CO_2 + H_2O_2$$

$$2\ H_2O_2 + \text{4-AAP} + \text{EHSPT} \xrightarrow{Peroxidase} \text{Quinoneimine} + 4H_2O$$

EHSPT = N-Ethyl-N-(2-Hydroxy-3-Sulfopropyl) m-Toluidine

4-AAP = Amino-4-antipyrine

The Samples were serum or heparinized plasma.

Reagents composition:

Reagent: R				
Phosphate buffer, pH 7.0			100	mmol/L
EHSPT			0.72	mmol/L
Ferrocyanide			0.03	mmol/L
Amino-4-antipyrine Uricase			0.37	mmol/L
	≥		150	U/L
Creatinine		≥	12 000	U/L

Reference values were 3-6 mg/dL (for women) in serum, plasma sample.

Procedure:

	Blank	Standard	Sample
Reagent R	200 µL	200 µL	200 µL
Distilled water	5 µL		
Standard or Sample		5 µL	5 µL

Mixed and the absorbance (A) was measured at 550 nm after 325 seconds incubation at 37 °C. Exactly 2 minutes after reading, the second reading was taken.

Calculation:

$(A_{sample} / A_{standard}) \times n$ n = standard concentration

Determination of serum Alanine aminotransferase (ALT/GPT)

Principle:

Kinetic determination of the Alanine aminotransferase (ALT) activity was done (Bergmeyer, 1985) according to the following reactions:

L-Alanine + α-Ketoglutarate $\xrightarrow{ALT}$ Pyruvate + L-Glutamate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-Lactate + NAD^+

LDH = Lactate dehydrogenase

The Samples were serum free from hemolysis or heparinized plasma.

Reagents composition:

Reagent 1: R1			
Tris buffer, pH 7.5 (30°C)		125	mmol/L
L-Alanine		680	mmol/L
LDH	≥	2 000	U/L
Reagent 2: R2			
NADH		1.1	mmol/L
α-Ketoglutarate		97	mmol/L

Reference values were 5-40 µ/L in serum, plasma sample.

Procedure:

	Blank	Standard	Sample
Reagent R1	200 µL	200 µL	200 µL
Reagent R2	50 µL	50 µL	50 µL
Sample			25 µL

Mixed and the change of absorbance per minute (ΔA/min) was measured at 340 nm between 50 seconds and 150 seconds incubation at 37 °C. The samples were read against distilled water.

Calculation:

Calculation was done at 340 nm, for a 1 cm light path cuvette according to the following formula:

$$\text{Activity (U/L)} = \Delta A/\text{min} \times 1746$$

Determination of serum Aspartate aminotransferase (AST/GOT)

Principle:

Kinetic determination of the Aspartate aminotransferase (AST) activity was done (Bergmeyer *et al.*, 1986) according to the following reactions:

$$\text{L- Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{AST} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{MDH} \text{L-Malate} + NAD^+$$

MDH = Malate dehydrogenase

Reagents composition:

Reagent 1: R1			
Tris buffer, pH 7.8 (30°C)		100	mmol/L
L-Aspartate		330	mmol/L
LDH	≥	2 000	U/L
MDH	≥	1 000	U/L
Reagent 2: R2			
NADH		1.1	mmol/L
α-Ketoglutarate		78	mmol/L

Reference values were 5-40 µ/L in serum, heparinized or EDTA plasma sample.

Procedure:

	Blank	Standard	Sample
Reagent R1	200 µL	200 µL	200 µL
Reagent R2	50 µL	50 µL	50 µL
Sample			25 µL

Mixed and the change of absorbance per minute (ΔA/min) was measured at 340 nm between 50 seconds and 150 seconds incubation at 37 °C. The samples were read against distilled water.

Calculation:

Calculation was done at 340 nm, for a 1 cm light path cuvette according to the following formula:

Activity (U/L) = $\Delta A/min \times 1746$

Determination of Alkaline phosphatase (ALP)

Principle:

In presence of Mg^{2+} and diethanolamine as phosphate acceptor, *p*-nitrophenylphosphate is transformed by alkaline phosphatases into phosphate and *p*-nitrophenol (yellow compound). Enzymatic-Kinetic determination of the alkaline phosphatase (ALP) activity was done according to the following reaction:

p-nitrophenylphosphate + $H_2O \xrightarrow{ALP}$ inorganic phosphate + *p*-nitrophenol

Reagents composition:

Reagent 1: R1		
Diethanolamine, pH 10.2	1.4	mol/L
Magnesium chloride	0.625	mmol/L
Reagent 2: R2		
p-nitrophenylphosphate	50	mmol/L

Reference values were 64-306 U/L in serum (for female).

Procedure:

	Blank	Standard	Sample
Reagent R1	250 µL	250 µL	250 µL
Reagent R2	60 µL	60 µL	60 µL
Sample			5 µL

Mixed and the change of absorbance per minute (ΔA/min) was measured at 405 nm between 50 seconds and 125 seconds incubation at 37 °C. The samples were read against distilled water.

Calculation:

Calculation was done at 340 nm, for a 1 cm light path cuvette according to the following formula:

$$\text{Activity (U/L)} = \Delta A/\text{ min} \times 3397$$

Determination of Bilirubin

Principle:

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of accelerator (cetrimide), conjugated and unconjugated bilirubin reacts with diazotized Sulfanilic acid to form azobilirubin (bilirubin total 4+1). In the absence of accelerator, only conjugated bilirubin reacts (Bilirubin direct 4+1). The increase of absorbance at 550 nm is proportional to bilirubin concentration. The determination of the bilirubin was done according to the following reactions (Sherwin, 2003):

Sulfanilic acid + $NaNo_2$ $\longrightarrow$ Diazotized Sulfanilic acid

Bilirubin + Diazotized Sulfanilic acid $\longrightarrow$ Azobilirubin

The Samples were serum free of hemolysis or heparinized plasma.

Reagents composition:

	Bilirubin	Bilirubin	Bilirubin	
	Total 4+1	Direct 4+1	Total & direct 4+1	
	Reagent 1: R1		Reagent 2: R2	
Sulfanilic acid	29	26	-	mmol/L
Hydrochloric acid	67	67	-	mmol/L
Cetrimide	37	-	-	mmol/L
Sodium nitrite	-	-	5.8	mmol/L

Reference values were 0.2-1.1 mg/dL for total bilirubin and < 0.25 mg/dL for direct bilirubin in serum, plasma sample.

Procedure:

	Calibration	Test
Reagent R1	160 µL	160 µL
Calibrator	20 µL	-
Sample	-	20 µL
Mixed and the absorbance (A1) was measured after 5 minutes incubation at 37 °C.		
Reagent R2	40 µL	40 µL

Mixed and the absorbance (A2) was measured at 550 nm after 5 minutes incubation at 37°C.

Calculation:

$[(A2\text{-}A1)_{sample} / (A2\text{-}A1)_{standard}] \times n$ n = calibrator concentration

Determination of serum glucose

Principle:

Enzymatic determination of glucose was done using the glucose oxidase (GOD) method (Burrin & Price, 1985; Trinder, 1969a, 1969b) according to the following reactions:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O$$

$$2\ H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

The Samples were serum free of hemolysis, plasma collected on fluoride or heparin/ iodoacetate or any inhibitors of glycolysis.

Reagents composition:

Reagent: R			
Phosphate buffer, pH 7.4		13.8	mmol/L
Phenol		10	mmol/L
4-aminoantipyrine		0.3	mmol/L
Glucose oxidase	≥	10000	U/L
Peroxidase	≥	700	U/L
Standard: Std			
D-Glucose	100	mg/dL	
	1	g/L	
	5.56	mmol/L	
ELITROL I	10×5	ml	
ELITROL II	10×5	ml	

Reference values were 70-110 mg/dL in serum, plasma sample.

Procedure:

	Blank	Standard	Sample
Reagent R	300 µL	300 µL	300 µL
Distilled water	3 µL		
Standard		3 µL	
Sample			3 µL

Mixed and the variation of absorbance (ΔA) was measured at 500 nm during 75 seconds incubation at 37 °C. The samples were read against reagent blank.

Calculation:

$(\Delta A_{sample} / \Delta A_{standard}) \times n$ n = standard concentration

Concentration value of Glucose standard 100 mg/dL is traceable to the standard reference material NIST 965a (of the national Institute of Standards and technology) (Barker *et al.*, 2006).

Determination of hematological parameters (complete blood count-CBC)

The samples were used for complete blood cell count (CBC) by automated hematology analyzer (Sysmex blood cell counter auto-analyzer model KX21; Japan). The analysis parameters include WBC (White Blood cell Count), RBC (Red Blood cell Count), HGB (hemoglobin), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), PLT (platelet), RDW-CV (RBC distribution width-coefficient of variation), PDW (platelet distribution width)*, and MPV (mean platelet volume).

The KX-21 employs three detector blocks and two kinds of reagents for blood analysis; The HGB detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method (volume or gram of hemoglobin in 1 dL of whole blood) (g/ dL). Non-cyanide hemoglobin analysis method rapidly converts blood hemoglobin as the Oxyhemoglobin method and contains no poisonous substance, making it suitable for automated method. Being capable of analysing methemoglobin, this method can accurately analyse control blood, etc. which contain methemoglobin.

The WBC count was measured by the WBC detector block using the DC (direct current) detection method (WBC count in 1 mL of whole blood) ($x10^3$ µL). Blood samples were aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance was changed, the blood cell size was detected as electric pulses. Blood cell count was calculated by counting the pulses.

The RBC count and platelets (PLT) were taken by the RBC detector block, also using the DC detection method (RBC count in 1 mL of whole blood) ($x10^6$ µL); and Platelet count in 1 mL of whole blood ($x10^3$ µL). The Hematocrit value was measured using the cumulative pulse height detection method (Ratio (%) of whole RBC volume in whole blood). The mean RBC volume (fL) was measured in whole blood, which is calculated by HCT/RBC. The mean RBC hemoglobin volume (pg) per RBC was calculated by HGB/RBC. The mean RBC hemoglobin concentration (g/dL), was calculated by HGB/HCT. The RBC distribution width – CV (RDW-CV) (%) was calculated from the points defining 68.26% of the entire area spreading from the peak of the RBC particle distribution curve. The Platelet distribution width (fL) was measured at the height of 20% from the bottom with the peak of platelet particle distribution curve taken as 100%. The mean platelet volume (fL) was measured as the mean volume of platelet.

For this experiment, the mode of analyzing collected blood sample was done in the whole blood status. Blood samples were heparinized by adding 1 drop (2 mg) EDTA to each 1 cc blood sample. The reagent used was WBC/HGB lyse-S III diff lytic reagent-PN 8546983 (STROMATOLYSER-WH).

For the WBC/HGB analysis, the blood samples were aspirated from the sample probe into the sample rotor valve one after another. 6 mL of blood measured by the sample rotor valve was transferred to the WBC transducer chamber along with 1.994 mL of diluent. At the same time, 1.0 mL of WBC/HGB lyse was added to prepare 1:500 dilution sample. When the solution was made to react in this status for approximately 10 seconds, RBC was hemolyzed and platelets shrink, with WBC membrane held as they were. At the same time, hemoglobin was converted into red coloured methemoglobin. Of the diluted/hemolyzed sample in the WBC transducer chamber, approximately 1 mL was

transferred to the HGB flow cell. 500 mL of sample in the WBC transducer was aspirated through the aperture. The pulses of the blood cells when passing through the aperture were counted by the DC detection method. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) was applied to the sample in the HGB flow cell. Concentration of this sample was measured as absorbance. This absorbance was compared with that of the diluent alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin value).

For analysis of RBC/PLT, blood was aspirated from the sample probe into the sample rotor valve. 4.0 mL of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 mL of diluent and brought to the mixing chamber as diluted sample (1st step dilution). Out of the 1:500 dilution samples, 40 mL is measured by the sample rotor valve, diluted into 1:25000 with 1.960 mL of diluent, and then transferred to the RBC/PLT transducer chamber (2nd step dilution). 250 mL of the sample in the RBC/PLT transducer chamber is aspirated through the aperture. At this time, RBC and PLT were counted by the DC detection method. At the same time, HCT (hematocrit value) was calculated by RBC pulse height detection method.

Other standard solutions used include: Latron Controls - PN 7546914, Latron Primer - PN 7546915, Calibration S-CAL - PN 7546808, 5C Cell control normal - PN 7546923 and 5C Cell Controls Tri Pack contains Normal, Abnormal I, and Abnormal II - PN 7547001. The detection limits were determined according to the Lab Protocol NHANES 2003–2004 (National Center for Health Statistics NHANES, 2004).

Reference values for females aged 19-65 years were as follows:

WBC	4-10	$\times 10^3$ µL
RBC	3.6-6.1	$\times 10^6$ µL
HGB	11.5-18.8	g/dL
HCT	34-54	%
PLT	150-450	$\times 10^3$ µL
MCV	80-100	fL
MCH	27-36	pg
MCHC	32-36	g/dL
RDW	11.6-14.6	%
PDW	7-20	fL
MPV	6-13	fL

Calculation of RBC Constant:

RBC constant (mean RBC volume, mean RBC hemoglobin, mean RBC haemoglobin concentration) were calculated from RBC, HGB, and HCT.

1. Mean RBC Volume (MCV): Calculation was made from RBC and HCT by the formula below:

$$MCV\ (fL) = HCT\ (\%) / RBC\ (\times 10^6/\mu L) \times 10$$

2. Mean RBC Hemoglobin (MCH): Calculation is made from RBC and HGB by the formula below:

$$MCH\ (pg) = HGB\ (g/dL) / RBC\ (\times 10^6/\mu L) \times 10$$

3. Mean RBC Hemoglobin Concentration (MCHC): Calculation is made from HCT and HGB by the formula below:

$$MCHC\ (g/dL) = HGB\ (g/dL) / HCT\ (\%) \times 100$$

Determination of specific gravity of urine

Principle:

The urine density test determines the concentration of ions in urine and shows a good correlation to the refractometrical method. The color of the test strip changes from deep blue in urine with low ionic concentration through green to yellow in urines with high ionic concentrations.

Reference values for evaluation of the results:

The test permits the determination of urine density between 1.000 and 1.030. Urines from adults with normal diets and normal fluid intake will have a density of 1.015-1.025. The chemical nature of the test strip may cause slightly different results from those obtained with other methods when elevated amounts of certain urine constituents are present; for example, the increase of urine density in dependence on glucose concentrations of > 1000 mg/dL (> 56 mmol/L) cannot be demonstrated by the strips. Elevated density readings maybe obtained in the presence of moderate quantities of protein. Highly buffered alkaline urines may cause low readings.

Reactive ingredients:

Bromothymol blue	12 µg
Copolymer	295 µg

Calculation:

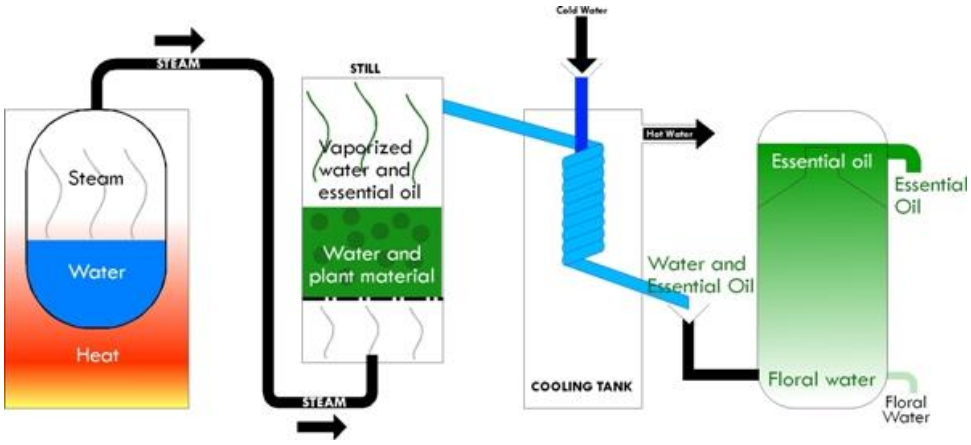
In any case, in order to establish a final diagnosis and prescribe an appropriate therapy, the results obtained with test strips should be verified with other medical results. The

effect of medicaments or their metabolic products on the test is not known in all cases. In case of doubt, it is recommended not to take the medicaments and then repeat the test.

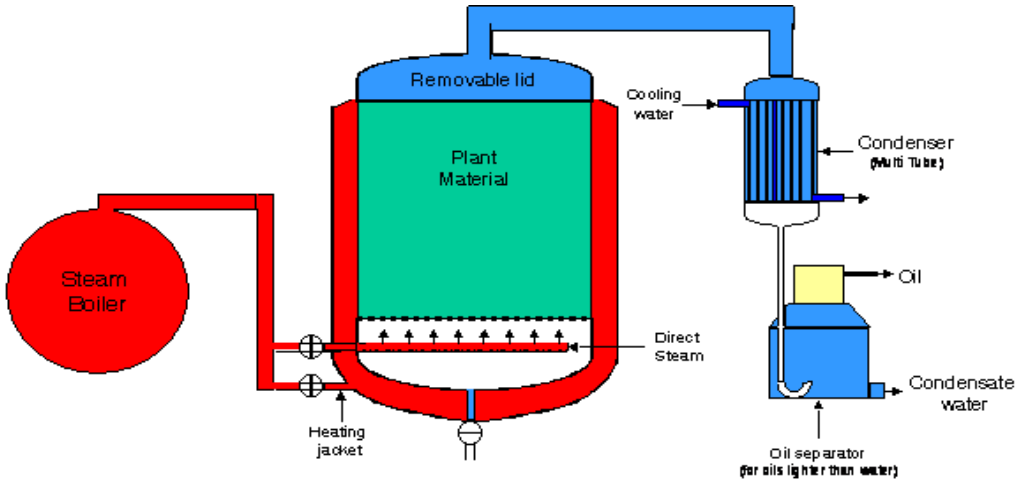
APPENDIX E: Procedure of caraway water extract production in Baharan factory

In general, steam distillation was conducted using a stainless still tank (tank A) which is the huge scale of clevenger apparatus. The caraway seeds were charged into the tank A from the top led using crane. Usually, the 2/3th of the tank A is filled with washed caraway seeds and water equally and 1/3th of the tank would be empty. The steam generated by the steam boiler (Rayiran and Garmiran, Iran) would be transferred into the tank A through steam lines and mixed with the caraway seeds. Further, the steam containing volatile compounds would be transferred into the connected condenser (Tank B) and the remaining caraway scum would be unloaded from the bottom of the tank. In tank B, the conveyed steam would be distilled into the water extract by cold water which is entrapped in the inner layer of the cooling tower placed on the top of the condenser. The remaining steam would be returned to the original tank by the steam trap and the condensed water extract would be transferred to the essence pitcher (Tank C- Schott Suprax Glass; Germany) through the water lines. During the distillation, the components would be vaporized and separated at different temperatures regardless of their solubility. However, the essential oil would be floated to the top of the essence pitcher and would be collected in the Petromax glass placed on the top of the still pitcher. The collected oil base product would be processed for export and the remaining water extract (herbal distillate) would be filtered, pumped into the storage tank and then filled into the PET bottles (1 or 4 liters). The final product would be labeled and dated to production and expire date and then stored in a dark store room before delivery to the market. The shelf life of the bottled water extract is around 1.5 year under suitable conditions (25 ^{o}C and with low humidity and light). From each 1 kg of caraway seeds, 10 liters caraway water extract was produced. Consequently, the amount of caraway in terms of w/v was 0.1 (10%) (3 gram caraway extract in 30 ml dose that was used) and, the distillation charge

defined as “the amount of plant material that can be processed in a single cycle” (Douglas *et al.*, 2006) would be around 300-400 kg. Further, the detection, isolation and structural determination of bioactive compounds present in the extracted caraway essential water such as polyphenol compounds (such as tannins and saponins) were assessed through one of the modern chemical analysis (GC-Mass).



APPENDIX E1: Diagram of the steam-distillation (Masango, 2005)



APPENDIX E2: Flow chart of the steam-distillation of caraway (Douglas *et al.*, 2006)

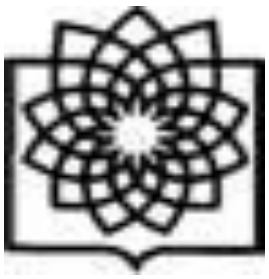
APPENDIX E3: Still tank

APPENDIX E4: Condenser (cooling tank)

APPENDIX E5: Oil separator tank (essence pitcher)

APPENDIX E6: Bottling and labelling of the product

APPENDIX F: Questionnaires



دانشگاه علوم پزشکی شهید بهشتی

انستیتو تحقیقات و دانشکده علوم تغذیه و صنایع غذایی

برگه رضایت آگاهانه کد شناسايي....

پروژه پژوهشی: بررسی اثر مصرف عرق زیره بر کاهش وزن زنان دارای اضافه وزن

اینجانب ..... رضایت خود را برای شرکت در پروژه تحقیقاتی فوق الذکر اعلام می دارم.

تمام اطلاعاتی که از اینجانب گرفته خواهد شد محرمانه باقی خواهد ماند. مشارکت داوطلبانه در اين پژوهش مي تواند در توليد اطلاعات جديد براى پيشگيري يا درمان چاقی کمک کند .من حق دارم در هر موقعي که نياز داشته باشم با محقق درباره مشكلات احتمالي که پيش مي آيد تماس بگيرم.

تاريخ : .....

امضاء : .....

آدرس فرد شرکت کننده در مطالعه:

تلفن ثابت (منزل/ محل کار): .....

تلفن همراه: .....

نظرات و پيشنهادات:

پرسشنامه اطلاعات عمومی

1. اطلاعات عمومی

سن . . . . . .شغل . . . . . رضایت شغلی . . . . . تحصیلات . . . . . وضعیت تاهل . . . .
. . . تعداد زایمان . . . سن اولین زایمان. . . . . نوع زایمان . . . . حامله . . . . . یائسه . . . . . شیرده

.تاریخچه پزشکی
- تاریخچه پزشکی در حال حاضر:
- سابقه پزشکی در گذشته:
- سابقه پزشکی خانواده:

(هر گونه مشکلات سلامتی مانند دیابت، سرطان، فشار خون بالا، چربی خون، بیماری های قلبی و عروقی، اختلال عملکرد غدد و متابولیک ,تیروئید، کبد، کلیه، کیسه صفرا، مثانه...)

2. اندازه گیری پارامترها

پارامترها	مقادیر
وزن(kg)	
قد (cm)	
BMI	
دور بازو (MUAC)	
دور کمر	
دور باسن	
دور کمر/ دور باسن (WHR)	
دور مچ دست	
دور ران	
فشار خون	
ضربان قلب	
توده استخوانی(kg)	
میزان سوخت و ساز پایه	
سرعت سوخت و ساز فعال	
درصد آب بدن٪	
توده چربی آزاد	
درصد توده چربی	
درصد عضله	

3. مصرف دارو

مصرف دارو یا مکمل (در حال حاضر و یا در 3 ماه گذشته)

- استفاده از داروهای موثر بر عملکرد نورواندوکرین، متابولیسم یا اشتها؟ (توضیح)
- مصرف هر گونه مکمل غذایی، چاقی، لاغری، ویتامین و مینرال ها (نام ببرید)

4. نوع فعالیت بدنی

یوگا ایروبیک پیاده روی غیره (شدت ، مدت، نوع)

5. وضعیت مصرف غذا و رژیم غذایی
- مصرف الکل سیگار غیره (نام ببرید)
- تعداد وعده ها ی اصلی میان وعده
- عادت به خوردن صبحانه
- داشتن رژیم های غذایی خاص: گیاه خوار خام گیاه خوار غذاهای آماده غیره
- سابقه آلرژی و حساسیت به ماده غذائی خاص (توضیح): سرد (آلرژی) گرم (کهیر)
- دچار مشکلات عصبی , پرخاشگری و عصبانیت حالت تهوع یبوست درد شکم نفخ
- آیا دچار کم اشتهائی پر اشتهائی یا تشنگی بیش از حد هستید؟
- تمایل به مصرف یا مصرف داروهای گیاهی خاص؟ (با ذکرعلت)
- میزان مصرف آب روزانه..... نوشیدنی گازدار..... چای/قهوه ..... غذاهای سرخ کردنی ..... نوع روغن ...

آیا تمایل به کاهش وزن دارید؟ هدف از کاهش وزن.....

6. نتیجه گیری

آزمون اشتها

:شماره کد شناسایی (VAS) مقیاس آنالوگ بصری برای امتیاز سیری و گرسنگی

1	2	3	4	5	6	7	8	9	10	اشتها
کاملا سیر									کاملا گرسنه	

لطفا شماره اشتهای خود را ا ز1 تا 10 (براساس جدول بالا) برای ۳ روزغیر متوالی هر 1 ساعت (از صبح تا شب) در جدول زیر یادداشت نمایید:

روز			روز		
ساعت	شماره اشتها	شماره اشتها	ساعت	ساعت	شماره اشتها
7 صبح					
8صبح					
9صبح					
10صبح					
11صبح					
12بعد از ظهر					
1بعد از ظهر					
2بعد از ظهر					
3بعد از ظهر					
4بعد از ظهر					
5بعد از ظهر					
6بعد از ظهر					
7شب					
8 شب					
9شب					
10شب					
11شب					
12شب					
میانگین					

آزمون پیتزا

لطفا معجون را مطابق دستور مصرف کنید (20 دقیقه قبل از غذا) صبحانه: یک کف دست نان ضخیم+ 1 تخم مرغ آب پز + 1 عدد گوجه ناهار (4 ساعت بعد از صبحانه): مینی پیتزای مخصوص تا زمانیکه کاملا سیر شوید. سپس تعداد قاچ های پیتزای خورده شده را یادداشت کنید. همچنین میزان اشتها و میزان میل به غذا را، هر یک ساعت شماره بزنید.

لطفا اگر شما با هر گونه عوارض جانبی خاص در طول دوره مواجه شدید ذکر کنید.

دانشگاه علوم پزشکی شهید بهشتی

انستیتو تحقیقات ودانشکده علوم تغذیه و صنایع غذایی

پرسشنامه بسامد خوراک 168 آیتمی

کد اقلام غذایی	مواد غذایی	مقدار	متوسط بار مصرف				ملاحضات
			روز	هفته	ماه	سال	
1	نان لواش	1کف دست					(تنوری، ماشینی)
2	نان بربری	1کف دست					
3	نان سنگک	1کف دست					
4	نان تافتون	1کف دست					
5	انواع نان های فانتزی	1عدد کوچک					نان باگت و ...
6	نان سبوس دار	1کف دست					(نان قهوه ای،جو...)
7	سایر نان ها	1کف دست					(سوخاری , قندی، روغنی، شیرمال،)
8	برنج پخته	1 بشقاب غ خ					
9	ماکاروني پخته	1کفگیر					
10	رشته (ورمشیل) پخته	1 لیوان					
11	رشته پخته	1 لیوان					
12	آرد گندم	یک استکان					
13	جو پخته	1ق،غ					
14	بلغور پخته	1ق،غ					
15	عدس	1ق،غ					
16	لوبیا (سفید،قرمز،چیتي)	1ق،غ					
17	نخود	1ق،غ					
18	باقلا پخته	1ق،غ					
19	سویا	1ق،غ					
20	ماش	1ق،غ					
21	لپه	1ق،غ					
22	گوشت گاو یا گوساله	1تکه خورشتي					
23	گوشت گوسفند	1تکه خورشتي					
24	گوشت چرخ کرده	1ق،غ					
25	مرغ و جوجه با پوست	1قطعه متوسط					
26	مرغ و جوجه بدون پوست	1قطعه متوسط					
27	ماهي با ذکر نوع آن	1قطعه متوسط					(به استثناي کنسرو تن)
28	تن ماهي(کنسرو)	قوطی1/2					
29	همبرگر	1عدد					
30	سوسیس	1عدد					
31	کالباس	1برش					
32	دل و جگر و قلوه	1تکه متوسط					
33	سیرابي و شیردان	1قطعه					

کد اقلام غذایی	مواد غذایی	مقدار	متوسط بار مصرف				ملاحضات
			روز	هفته	ماه	سال	
34	زبان	1عدد کامل					
35	مغز	1عدد کامل					
36	کله	1عدد کامل					
37	پاچه	1عدد					
38	تخم مرغ	1عدد					
39	شیر بدون چربي/کم چرب	1ليوان					(2/5%>)
40	شیر با چربی معمولی	1ليوان					(2/5%>)
41	شیر پرچرب	1ليوان					(2/5%<)
42	شیر کاکائو	1شيشه تجاري					
43	شیر شکلاتي	1شيشه تجاري					
44	ماست چکیده	1ق،غ					
45	ماست کم چرب/ معمولي	کاسه کوچک					
46	ماست پرچرب	کاسه کوچک					
47	ماست خامه اي	1ق،غ					
48	پنیر	1قوطي کبريت					(به استثناي خامه اي و ليقوان)
49	پنیر خامه اي/ليقوان	1قوطي کبريت					
50	دوغ	1ليوان					
51	خامه و سر شیر	1ق،غ					
52	بستني سنتي	نصف ليوان					
53	بستني غيرسنتي	نصف ليوان					
54	کره	1قوطي کبريت					آنچه که به غذا افزوده میشود +
55	مارگارین	1قوطي کبريت					آنچه که به غذا افزوده میشود +
56	کشک	1ق،غ					
57	کاهو خرد شده	نصف ليوان					
58	گوجه فرنگي	1عدد متوسط					
59	خیار	1عدد متوسط					
60	سبزي خوردن	1پيش دستي					
61	سبزي (پخته)	نصف ليوان					خورشتی/آش/کوکو
62	کدو حلوايي	قطعه 6×6					
63	کدو خورشتي	1عدد متوسط					
64	بادمجان پخته	1عدد متوسط					
65	کرفس پخته	نصف ليوان					
66	سیب زمینی	1عدد متوسط					
67	سیب زمینی سرخ کرده	10 خلال متوسط					
68	نخود سبز پخته	نصف ليوان					
69	لوبیا سبز پخته	1ق،غ					
70	هویج خام	1عدد متوسط					
71	هویج پخته	1عدد متوسط					
72	سیر	1حبه					
73	پیاز خام	1عدد متوسط					
74	پیاز سرخ شده	1ق،غ					
75	کلم و انواع آن	کاسه ماست خوری					(قرمز،قمری، گل کلم، بروکلی سفید،)
76	فلفل دلمه اي	1عدد متوسط					

کد اقلام غذایی	مواد غذایی	مقدار	متوسط بار مصرف				ملاحضات
			روز	هفته	ماه	سال	
77	اسفناج خام	20 برگ متوسط					
78	اسفناج پخته	نصف لیوان					
79	شلغم	1عدد متوسط					
80	قارچ پخته	نصف لیوان					
81	فلفل سبز	1عدد متوسط					
82	ذرت و بلال	1عدد متوسط					
83	رب گوجه فرنگی/سس قرمز	1ق،غ					
84	ترشی (با ذکر محتویات)	1ق،غ					
85	شور (با ذکر محتویات)	1ق،غ					
86	خیار شور	1عدد متوسط					
87	طالبی	طالبی متوسط1/4					
88	خربزه	1قاچ متوسط					
89	هندوانه	1قاچ متوسط					
90	گلابي	1عدد متوسط					
91	زردآلو	1عدد متوسط					
92	گیلاس	1عدد متوسط					
93	سیب	1عدد متوسط					
94	هلو	1عدد متوسط					
95	شلیل	1عدد متوسط					
96	گوجه سبز	1عدد متوسط					
97	انجیر تازه	1عدد متوسط					
98	انجیر خشک	1عدد متوسط					
99	انگور	1خوشه کوچک					
100	کیوي	1عدد متوسط					
101	گریپ فروت	1عدد متوسط					
102	پرتقال	1عدد متوسط					
103	خرمالو	1عدد متوسط					
104	نارنگي	1عدد متوسط					
105	انار	1عدد متوسط					
106	خرما	1عدد متوسط					
107	آلو(زرد و قرمز)	1عدد متوسط					
108	آلبالو	10عدد متوسط					
109	توت فرنگي	3عدد متوسط					
110	موز	1عدد متوسط					
111	لیمو شیرین	1عدد متوسط					
112	لیمو ترش	1عدد متوسط					
113	آب گریپ فروت	1لیوان					
114	آب پرتقال	1لیوان					
115	آب سیب	1لیوان					
116	آب طالبی	1لیوان					
117	ذغال اخته	1لیوان					
118	آناناس تازه	1لیوان					
119	آناناس کنسرو	1لیوان					

کد اقلام غذایی	مواد غذایی	مقدار	متوسط بار مصرف				ملاحضات
			روز	هفته	ماه	سال	
120	کشمش	1ق،غ					
121	گرمک	کاسه ماست خوري					
122	توت تازه	10عدد متوسط					
123	توت خشک	20عدد متوسط					
124	برگه هلو	10عدد متوسط					
125	برگه زردآلو	10عدد متوسط					
126	کمپوت میوه جات	-					(با ذکر نوع و مقدار)
127	زیتون سبز	10عدد متوسط					
128	روغن نباتي جامد	1ق،غ					
129	روغن مایع	1ق،غ					(...آفتاب گردان,ذرت ,سویا،)
130	روغن زیتون	1ق،غ					
131	پیه	یک تکه متوسط					
132	روغن حیواني	1ق،غ					
133	سس مایونز	1ق،غ					
134	بادام زميني	20عدد مغز					
135	بادام	10عدد مغز					
136	گردو	1عدد مغز کامل					
137	پسته	10عدد					
138	فندق	10عدد					
139	تخمه	کاسه کوچک					(کدو،آفتاب گردان،هندوانه)
140	بیسکویت(نوع و اندازه)	-					
141	کراکر	1عدد					
142	کیک یزدي	1عدد					
143	کیک خانگي(تولد و...)	1برش متوسط					
144	سایر کیک ها و کلوچه	1عدد					
145	شیریني خشک	1عدد متوسط					
146	شیریني تر	1عدد متوسط					
147	چاي	1لیوان					
148	قند یا شکر پنیر	10حبه					
149	شکر	1ق،م					
150	حلوا شکري	عدد1/4					
151	عسل	1ق،م					
152	مربا(با نوع)	1ق،غ					نوع:.....
153	شکلات/کاکائو/تافی	1عدد					با ذکر نوع
154	آبنبات	1عدد					
155	نبات	1تکه متوسط					
156	پفک	1بسته					
157	چیپس	1بسته					ذکر تعداد حلقه
158	گز	1عدد متوسط					آردی/ لقمه ای
159	سوهان	1عدد					
160	نقل	10عدد					
161	کرم کارامل	1ق.غ					
162	حلوا خانگی	1ق.غ					نوع:.....

کد اقلام غذایی	مواد غذایی	مقدار	متوسط بار مصرف				ملاحضات
			روز	هفته	ماه	سال	
163	پیراشکی	1عدد					
164	قهوه / نسکافه	1لیوان					
165	نوشابه گازدار	1بطری					رژیمی/معمولی
166	آبمیوه صنعتی	1بسته/لیوان					
167	آبلیمو/آبغوره	1ق.م					
168	نمک	1ق.م					

دانشگاه علوم پزشکی شهید بهشتی

دانشکده علوم تغذیه و صنایع غذایی

پرسشنامه فعالیت بدنی

نام و نام خانوادگی کدشناسايي

پروژه پژوهشی: بررسی اثر مصرف عرق زیره بر کاهش وزن زنان دارای اضافه وزن

فعالیتهای مختلف	دقیقه	ساعت	جمع زمان	MET
خواب- استراحت	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		۰/۹
نشستن- تماشای تلویزیون-مطالعه	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		۱
فعالیت های رایانه ای، نشستن در کلاس یا جلسه	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		۱/۵
فعالیت های روزمره خانه ، رانندگی	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		۲
تمیزکاری(گردگیری، جاروکردن) - راه رفتن آرام (پیاده روی)	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		۳
دوچرخه سواری - پیاده روی تند	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		۴
بالا رفتن از پله با حمل اشیای سبک	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		۵
باغبانی - نرمش و ایروبیک	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		۶
انجام فعالیت های ورزشی از قبیل دو، والیبال و غیره	□□□ ۱۵ ۳۰ ۴۵	□□□□□□□□□□ ۱ ۲ ۳ ۴ ۵ ۶ ۷ ۸ ۹ ۱۰		>۶

در صورت شاغل بودن:				
کار/شغل: به‌صورت اغلب نشسته	□□□ 15 30 45	□□□□□□□□□□ 1 2 3 4 5 6 7 8 9 10		1/3
کار/شغل: به‌صورت نیمی از ساعات نشسته	□□□ 15 30 45	□□□□□□□□□□ 1 2 3 4 5 6 7 8 9 10		1/8
کار/شغل: به‌صورت اغلب ایستاده	□□□ 15 30 45	□□□□□□□□□□ 1 2 3 4 5 6 7 8 9 10		2/2
کار/شغل: به‌صورت اغلب در حال راه رفتن، بلند کردن و حمل بار سبک	□□□ 15 30 45	□□□□□□□□□□ 1 2 3 4 5 6 7 8 9 10		2/6
کار/شغل:به‌صورت اغلب در حال راه رفتن، بلند کردن و حمل بار سنگین	□□□ 15 30 45	□□□□□□□□□□ 1 2 3 4 5 6 7 8 9 10		3
کار/شغل: به‌صورت کار دستی سنگین	□□□ 15 30 45	□□□□□□□□□□ 1 2 3 4 5 6 7 8 9 10		3/9

AGRREEMWENT LETTER

Research Topic: Evaluating the effect of caraway water extract intake on overweight and obese women

I ……..…..hereby, agree to attend in this research work.

All the information taken will remain confidential. Participating in this research work could be helpful in providing new information for preventing and treatment of obesity.

I have the right to contact with the researcher whenever I have problems regarding this research work.

Date:…….

Signature:…….

Address:…… …….

Phone number: ……..

Suggestions:…… ……..

1- GENERAL INFORMATION QUESTIONNAIRE

I. GENERAL INFORMATION

Name_____ Age ___Gender _____Occupation _____Education

Marital status _____ age of first delivery _____ number of delivery_____

Type of delivery_____ Pregnant _____Breast-feeding_____Menopause_____

II. MEDICAL HISTORY

-Present medical history: _____

-Past medical history: _____

-Family history: _____

(Any health problems such as diabetes, cancer, high blood pressure, hyperlipidemia, cardiovascular diseases, hyper/hypo thyroid, and liver, kidney, gall bladder, bladder,…. dysfunction)

III. BIOMEDICAL and ANTHROPOMETRIC MEASUREMENTS

Parameters	Values
Weight	
Height	
BMI	
MUAC	
Waist circumference	
Hip circumference	
WHR	
Wrist circumference	
BP (blood pressure)	
HR (heart rate)	
BM (bone mass)	
BMR (basic metabolic rate)	
AMR (active metabolic rate)	
Body water%	
FFM (fat free mass)	
FM (fat mass percentage)	
Muscle percentage	

IV. **MEDICATIONS**

Indicate any medications, dietary or weight loss supplements you're currently taking or have taken in the last three months:

Other medications and dosages (if known):

Name of medication	Reason for taking and for how long have you been taking this

V. PHYSICAL ACTIVITY (H / WK)

Yoga, aerobics, walking, etc. (intensity, duration, type)

VI. FOOD INTAKE STATUS

Smoking, alcohol consumption, etc. (specify)

No of meals / day ______ 1 □ 2 □ 3 □ ≥

Breakfast skipper----------

Any special dietary habits: Veg □ non veg □ mixed □

Food allergy – yes/ no if yes specify _____

Food fads – yes/ no if yes specify _____

Nausea – yes /no, vomiting –yes/no, anorexia-yes/no, constipation - yes/no

Are you interested to take or taking any specific herbal supplement?

Are you interested in losing weight? Why?

VII. CONCLUSION

APPETITE TESTS

Visual analogue scale (VAS) for rating fullness and hunger Identical code

Appetite	10	9	8	7	6	5	4	3	2	1
	Extremely hungry									Extremely full

Please note your appetite code from 1 to 10 (based on the above table) for 3 non-consecutive days every 1 hour (from morning till night), in the following table:

Day		Day		Day	
Hour	Appetite code	Hour	Appetite code	Hour	Appetite code
7 AM					
8 AM					
9 AM					
10 AM					
11 AM					
12 PM					
1 PM					
2 PM					
3 PM					
4 PM					
5 PM					
6 PM					
7 PM					
8 PM					
9 PM					
10 PM					
11 PM					
12 PM					
Average					

Pizza Test:

Please eat the provided preparation according to the instructions (20 minutes before meals)

Breakfast: a palm-size thick bread + 1 boiled egg + 1 tomato
Lunch (4 hours after breakfast): mini pizza until you are completely satisfied. Then write down the number of pizza slices eaten. Also, write down your appetite code every hour.

Please mention if you encountered any specific side-effects during intervention period:

2- FOOD FREQUENCY QUESTIONNAIRE (168-item FFQ)

Code	Food item	Amount/size	Average times consumed				Descriptions
			Day	Week	Month	Year	
1	Lavash bread	1 Palm-sized					
2	Barbari bread	1 Palm-sized					
3	Sangak bread	1 Palm-sized					
4	Taftoon bread	1 Palm-sized					
5	Baguette bread	1 small					
6	Whole meal bread	1 Palm-sized					Brown bread , barley bread,…
7	Other breads	1 Palm-sized					Sugary, oily, milky, toasted,..
8	Cooked rice	1 plate/dish					
9	Cooked spaghetti	skimmer 1					
10	Cooked noodle	1 cup					
11	Cooked reshteh	1 cup					
12	Wheat flour	1 cup					
13	Cooked barley	1 tablespoon					
14	cooked grits	1 tablespoon					
15	Lentils	1 tablespoon					
16	beans)white, red, pinto(	1 tablespoon					
17	Peas	1 tablespoon					
18	Cooked beans (baghela)	1 tablespoon					
19	Soya	1 tablespoon					
20	Mash	1 tablespoon					
21	Cotyledon	1 tablespoon					
22	Beef	1 stewed piece					
23	Lamb	1 stewed piece					
24	Minced meat	1 tablespoon					
25	chicken with the skin	1 medium piece					
26	chicken without skin	1 medium piece					
27	Fish	1 medium piece					Excluding canned tuna
28	canned tuna fish	1//2 can					
29	Burger	1					
30	Sausage	1					
31	Kielbasa	1 slice					
32	Heart, liver and kidney	1 medium piece					
33	Sirabi and shirdan	1 piece					
34	Tongue	1 full					
35	Maghz	1 full					
36	Kale	1 full					
37	Paache	1 full					
38	egg	1 full					
39	Skimmed/low fat milk	1 glass					(2/5%>)
40	Milk with normal fat	1 glass					(2/5%>)

Code	Food item	Amount/size	Average times consumed				Descriptions
			Day	Week	Month	Year	
41	Whole milk	1 glass					(2/5%<)
42	Cocoa milk	1 bottle					
43	Chocolate milk	1 bottle					
44	Chekideh yogurt	1 tablespoon					
45	Low fat yogurt	1 small bowl					
46	High fat yogurt	1 bottle					
47	Creamy yogurt	1 tablespoon					
48	Feta/white Cheese	1 match-box					
49	Creamy cheese/Lighvan	1 match-box size					
50	Doogh/buttermilk	1 glass					
51	Cream and sarshir	1 tablespoon					
52	Sonati ice cream	½ glass					
53	Packed ice cream	½ glass					
54	Butter	1 match-box size					
55	Margarine	1 match-box size					
56	Kashk	1 tablespoon					
57	Shredded lettuce	½ cup					
58	tomato	1					
59	Cucumber	1					
60	Preemption herbs	1 small plate					
61	Cooked vegetables	½ cup					
62	pumpkin	Piece 6×6					
63	zucchini	1 medium					
64	eggplant cooked	1 medium					
65	Cooked celery	½ cup					
66	Potato	1 medium					
67	French fries	10 medium					
68	Cooked green peas	½ cup					
69	Cooked green beans	1 tablespoon					
70	Raw carrot	1 medium size					
71	Cooked carrot	1 medium size					
72	garlic	1 clove					
73	Raw onion	1 medium size					
74	Fried onion	1 tablespoon					
75	cabbage	A small bowl					cauliflower, red, broccoli, white,…
76	sweet pepper	1 medium					
77	raw spinach	20 medium leaves					
78	Cooked spinach	½ cup					
79	turnip	1 medium					
80	cooked mushrooms	½ cup					
81	green pepper	1 medium					
82	corn	1 medium size					

Code	Food item	Amount/size	Average times consumed				Descriptions
			Day	Week	Month	Year	
83	Ketchup/ tomato sauce	1 tablespoon					
84	pickle	1 tablespoon					
85	Sauerkraut	1 tablespoon					
86	Pickled cucumber	1 medium size					
87	cantaloupe melon	¼ full					
88	melon	1 medium slice					
89	watermelon	1 medium slice					
90	Pear	1 medium size					
91	Apricot	1 medium size					
92	cherry	1 medium size					
93	Apple	1 medium size					
94	Peach	1 medium size					
95	Nectarine	1 medium size					
96	Greengage	1 medium size					
97	fresh figs	1 medium size					
98	dried figs	1 medium size					
99	Grapes	1 small bunch					
100	Kiwi	1 medium size					
101	Grapefruit	1 medium size					
102	Orange	1 medium size					
103	Persimmons	1 medium size					
104	Tangerine	1 medium size					
105	Pomegranate	1 medium size					
106	Dates	1 medium size					
107	Plum	1 medium size					
108	Black cherry	1 medium size					
109	Strawberries	1 medium size					
110	Banana	1 medium size					
111	sweet lime	1 medium size					
112	Lemon	1 medium size					
113	grapefruit juice	1 glass					
114	orange juice	1 glass					
115	apple juice	1 glass					
116	Cantaloupe juice	1 glass					
117	Blueberries	1 glass					
118	fresh pineapple	1 glass					
119	canned pineapple	1 glass					
120	Raisins	1 tablespoon					
121	Honeydew	1 small bowl					
122	Fresh mulberry	10 medium size					
123	dried mulberry	20 medium size					
124	Dried peach	10 medium size					
125	Dried apricot	10 medium size					

Code	Food item	Amount/size	Average times consumed				Descriptions
			Day	Week	Month	Year	
126	Canned fruits	-					Type and amount
127	Olives	10 medium size					
128	Hydrogenated vegetable oil	1 tablespoon					
129	vegetable oil	1 tablespoon					Rice bran, soy, sunflower, Corn
130	olive oil	1 tablespoon					
131	Tallow	1 medium piece					
132	Ghee	1 tablespoon					
133	Mayonnaise	1 tablespoon					
134	Peanut	20					
135	Almonds	10					
136	Walnuts	1 full					
137	Pistachios	10					
138	Hazelnuts	10					
139	Tokhme (Seeds)	Small bowl					Pumpkin , sunflower , watermelon
140	Biscuit	1 medium size					
141	Cracker	1					
142	Yazdi Cake	1					
143	homemade cake	1 medium slice					
144	Cookies	1					
145	Dry sweets`	1 medium size					
146	Creamy sweets	1 medium size					
147	Tea	Cup					
148	sugar cubes	10 cubes					
149	Sugar	1 teaspoon					
150	Sesame halva	¼ pieces					
151	Honey	1 teaspoon					
152	Jam	1 tablespoon					
153	Chocolate/Cocoa/toffee	1					
154	Candy	1					
155	Nabat	1 medium piece					
156	Snack	1 pack					
157	Chips	1 pack					
158	Gaz	1					
159	Sohan	1					
160	Noghl	10					
161	Creamy caramel	1 tablespoon					
162	Home-made halva	1 tablespoon					
163	Donuts	1					
164	Coffee/Nescafe	1 glass					
165	Soda	1 bottle					
166	Juice	1 glass/pack					

Code	Food item	Amount/size	Average times consumed				Descriptions
			Day	Week	Month	Year	
167	Lemon juice	1 teaspoon					
168	Salt	1 tablespoon					

3- PHYSICAL ACTIVITY LEVEL QUESTIONNAIRE

Type of activity	No. of days	Total time Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋	MET
Sleeping-resting, reclining			0.9
Sitting, watching TV, reading			1
Computer activities, sitting in the class, …			1.5
Driving, daily house work & domestic chores			2
Cleaning the house (dusting, sweeping,…), slow walking			3
Moderate-intensity sports such as cycling, brisk walking			4
Carrying/moving moderate loads, climb up the stairs			5
Gardening, aerobics,….			6
Vigorous-intensity sports such as running, fitness & games (volleyball, fast swimming, …)			> 6
IF WORKING OR EMPLOYMENT			
Sitting frequently			1.3
Half of time sitting			1.8
Standing frequently			2.2
Walking, carrying moderate loads frequently			2.6
Walking, carrying heavy loads frequently			3
Heavy working frequently, carrying or lifting heavy loads, digging or construction work			3.9

APPENDIX G: The first page of the publications and papers presented

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2013, Article ID 928582, 8 pages
http://dx.doi.org/10.1155/2013/928582

Hindawi

Research Article

Antiobesity Effect of Caraway Extract on Overweight and Obese Women: A Randomized, Triple-Blind, Placebo-Controlled Clinical Trial

Mahnaz Kazemipoor,[1] Che Wan Jasimah Bt wan Mohamed Radzi,[1] Majid Hajifaraji,[2] Batoul Sadat Haerian,[3] Mohammad Hossein Mosaddegh,[4] and Geoffrey A. Cordell[5]

[1] *Department of Science & Technology Studies, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[2] *National Nutrition & Food Technology Research Institute, Faculty of Nutrition & Food Technology, Shahid Beheshti University of Medical Sciences, Tehran 1981619573, Iran*
[3] *Department of Pharmacology, Faculty of Medicine, University of Malaya, 60302 Kuala Lumpur, Malaysia*
[4] *Department of Pharmacology and Toxicology, Pharmacy School, Yazd Shahid Sadoughi Medical Sciences University, Yazd 8917945556, Iran*
[5] *Natural Products Inc., Evanston, IL 60203, USA*

Correspondence should be addressed to Mahnaz Kazemipoor; mahnaz.kp@gmail.com

Received 18 July 2013; Accepted 5 September 2013

Academic Editor: James William Daily III

Caraway (*Carum carvi* L.), a potent medicinal plant, is traditionally used for treating obesity. This study investigates the weight-lowering effects of caraway extract (CE) on physically active, overweight and obese women through a randomized, triple-blind, placebo-controlled clinical trial. Seventy overweight and obese, healthy, aerobic-trained, adult females were randomly assigned to two groups ($n = 35$ per group). Participants received either 30 mL/day of CE or placebo without changing their diet or physical activity. Subjects were examined at baseline and after 90 days for changes in body composition, anthropometric indices, and clinical and paraclinical variables. The treatment group, compared with placebo, showed a significant reduction of weight, body mass index, body fat percentage, and waist-to-hip ratio. No changes were observed in lipid profile, urine-specific gravity, and blood pressure of subjects. The results suggest that a dietary CE with no restriction in food intake, when combined with exercise, is of value in the management of obesity in women wishing to lower their weight, BMI, body fat percentage, and body size, with no clinical side effects. In conclusion, results of this study suggest a possible phytotherapeutic approach for caraway extract in the management of obesity. This trial is registered with NCT01833377.

1. Introduction

Proper nutrition is necessary to keep the body healthy and functioning normally. The addition of extra calories in the diet induces fat accumulation, leading to overweight and obesity. According to the World Health Organization (WHO), excess body weight and obesity are recognized as a body mass index (BMI) greater than 25 kg/m^2. According to this report by WHO, "globesity," as a foodborne illness, is a rapidly growing global problem, which is maximizing the risk of various health problems, such as type 2 diabetes, cardiovascular diseases (CVD), musculoskeletal disorders, and cancer. Overweight and obesity are associated with high morbidity and mortality, resulting in considerable health care costs and other economic and social impacts on the society. Since 1980, obesity has almost doubled worldwide and is recognised as one of the leading causes of death. In 2008, over 1.4 billion adults, predominantly women, were overweight or obese. Finally, more people die because of being overweight and obese than those who are underweight, and this disease state is the fifth main reason for mortality and the sixth for health problems globally. Management of obesity is therefore a public health necessity [1–5].

PHYTOTHERAPY RESEARCH
Phytother Res. (2014)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5147

Preliminary Safety Evaluation and Biochemical Efficacy of a *Carum carvi* Extract: Results from a Randomized, Triple-Blind, and Placebo-Controlled Clinical Trial

Mahnaz Kazemipoor,[1]* Che Wan Jasimah Bt Wan Mohamed Radzi,[1] Majid Hajifaraji[2] and Geoffrey A. Cordell[3]

[1]Department of Science and Technology Studies, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
[2]National Nutrition and Food Technology Research Institute, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran 1981619573, Iran
[3]Natural Products Inc., Evanston, IL 60203, USA

Carum carvi* L. (Apiaceae) is known as caraway, and its derivatives find wide medicinal use for health purposes, including for gastrointestinal problems and obesity. Since there is inconsistency among the reports on the safety of this plant in humans, this research was aimed at assessing the safety of a characterized caraway aqueous extract (CAE) in a randomized, triple-blind, placebo-controlled study. Seventy, overweight and obese, healthy women were randomly assigned into placebo ($n = 35$) and plant extract ($n = 35$) groups. Participants received either 30 ml/day of CAE or placebo. Subjects were examined at baseline and after 12 weeks for changes in heart rate, blood pressure, urine test, 25-item blood chemistries, and general health status. No significant changes of blood pressure, heart rate, urine specific gravity, and serum blood tests were observed between the two groups before and after treatment. However, in the complete blood count test, red blood cell levels were significantly ($p < 0.01$) increased, and platelet distribution width was significantly decreased after the dietary CAE treatment, as compared with placebo. No negative changes were observed in the general health status of the two groups. This preliminary study suggests that the oral intake of CAE appears to be without any adverse effects at a dosage of 30 ml daily for a period of 12 weeks. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: Carum carvi; caraway aqueous extract; volatile compounds; safety; obesity; phytotherapy.

INTRODUCTION

Primary health care based on plant extracts, or isolated natural products, is a popular and sometimes the only treatment approach available to communities all over the world. Indeed, approximately 25% of modern prescription medicines are derived from plants (Farnsworth *et al.*, 1985). Plant-based medications are frequently perceived as being a natural and harmless (safe) form of therapy. However, medicinal plants and their extracts contain numerous phytochemicals, including toxins of exceptional potency, which target a diverse array of human genomic sites. Consequently, their consumption might result in a range of unpleasant reactions, or cause interactions, synergistic or antagonistic, with other medications. Therefore, scientifically establishing the safety of the constituents in a particular preparation must be a primary consideration in the delivery of phytotherapeuticals (Cordell and Colvard 2012). It is important to acknowledge that the safety of a plant cannot be assumed based on historical usage, especially if a different plant part or extraction methodology is employed. In addition, there is significant evidence accumulating to show that the ingestion of some plant-based products causes side effects and adverse reactions (Calixto 2000; Ernst 1998; Kazemipoor *et al.*, 2012). Thus, characterization of a plant preparation being biologically evaluated is critical for reproducibility.

Caraway, *Carum carvi* L. (Apiaceae), is a common and ancient medicinal plant. It is recommended as being safe in the form of a spice, as an extract, or as an essential oil, or in combination with other phytochemicals, for the treatment of a range of health problems (Sadowska and Obidoska 2003; Westphal *et al.*, 1996). In addition, a variety of *in vitro* and *in vivo* studies have examined a range of different biological and pharmacological activities and health benefits of caraway ingredients. Several animal and human studies have suggested the efficacy of caraway preparations in the treatment of a number of diseases, including gastrointestinal problems (Khayyal *et al.*, 2001; Thompson Coon and Ernst 2002), hyperglycemia (Ene *et al.*, 2007), cancer (Kamaleeswari *et al.*, 2006; Mazaki *et al.*, 2006), and obesity (Kazemipoor *et al.*, 2013).

A number of studies have also reported on the safety and tolerability of caraway, along with its hepatoprotective potential (Sadowska and Obidoska 2003; Samojlik *et al.*, 2010; Westphal *et al.*, 1996). Therefore, caraway, by having a promising safety profile, has been recommended as an encouraging ingredient for safe usage as a traditional

* Correspondence to: Mahnaz Kazemipoor, Department of Science and Technology Studies, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia.
E-mail: mahnaz.kp@gmail.com

Received 08 November 2013
Revised 21 February 2014
Accepted 25 February 2014

Safety, Efficacy and Metabolism of Traditional Medicinal Plants in the Management of Obesity: A Review

Mahnaz Kazemipoor, Che Wan Jasimah Wan Mohamed Radzi, Geoffrey A. Cordell, and Iman Yaze

***Abstract*—Obesity is an important global health concern, and is associated with high morbidity and mortality rates. Modern methods of treatment, such as synthetic drugs and surgery, still have to be improved to show safety and efficacy. The main concerns with such treatments are the high costs and serious complications. As a result, there is great interest in the use of plant-based medicinal agents as an alternative therapy. This study aims to provide a review of the studies on accessible botanical sources for the treatment of obesity. Based on published studies, this review attempts to explain how these medicinal plants act in humans to cause weight loss, and which method of usage is safer and more efficient. Information was gathered from books, journals and electronic sources published in the period of 1991 to 2012. The medicinal plants studied can reduce weight through five basic mechanisms, including stimulating thermogenesis, lowering adipogenesis, enhancing lipolysis, suppressing appetite, and decreasing the absorption of lipids. Furthermore, consumption of reliable medicinal plant extracts in a single form and at an optimum dosage could be a safe treatment for obesity. However, based on reviews, some combinations of certain medicinal plantsmay result in either lower efficacy or cause unexpected side-effects.**

***Index Terms*—Anti-obesity, fat absorption, slimming aids, dietary supplements**

I. Introduction

Since 1997, the WHO has warned of obesity as a global epidemic, although it was not noticeable during most of the 20th century [1]-[2]. Statistics show that the prevalence of obesity had reached up to 400 million adults by 2005 [3]. Currently, it is reported that half of the adult population in OECD countries are overweight, and 1/6th are obese. Based

on the classification by WHO a body mass index [BMI] greater than 25 Kg/m2 is defined as overweight and BMI value of greater than 30 Kg/m^2 is termed as obesity, besides, BMI greater than 40 Kg/m2 is termed "morbid obesity. According to this report by WHO, obesity is related to several health problems, including cardiovascular diseases, hypertension, diabetes mellitus, gallbladder disease, cancer, endocrine and metabolic disturbances, osteoarthritis, gout, pulmonary diseases, as well as psychological problems such as social bias, prejudice, discrimination, body shape dissatisfaction, and eating disorders which are seen in both children and adults [4]. From an economic point of view, obesity and its related health consequences involve enormous costs currently and for future health care, such as physician visits, hospitalization, and other related expenses [5]-[6]-[7]. Being overweight is a cosmetic problem and a major risk factor for human health [8]. In short, obesity can cause a decline in life expectancy [9]. Despite vast attempts to address this issue, "globesity" remains an enormous challenge.

A. Challenges in Treating Obesity

Recently, there has been a proliferation of different anti-obesity products appearing on the market [10]. Despite the high cost of such products, their long-term consumption is still not recommended as they have exhibited several side effects, such as gastrointestinal and kidney problems [11]. For example, among the varieties of anti-obesity drugs, only Orlistat and Sibutramine can be used long-term. In addition, such products do not satisfactorily impact weight loss or are not tolerated by the body [12]-[13].

However, the use of natural remedies for weight loss has increased. Scientists believe that botanical sources seem more reliable, safer, and also cheaper than current conventional methods, such as synthetic chemical drugs [14] or surgical procedures [15] which may have adverse effects or be of limited duration in effectiveness [16].

B. Natural Medications

Studies show that natural food ingredients and medicinal plant preparations are able to enhance satiety, boost metabolism, and speed up weight loss [17]-[18]. Including these foods in the diet on a regular basis will therefore assist an individual to lose weight slowly. However, there is still some doubt about their application for humans [19]. On the other hand, despite the global market for satiety, fat burning, dietary supplements and other weight management remedies, the awareness of the usefulness of these products is neither sufficient nor clearly perceived by patient [20]. This study aims to provide a review of previous reports about the availability of natural medicinal agents and their potential for assisting in losing weight. This information could aid patients in their selection of the appropriate botanical product to develop a lean and healthy body.

II. Methods of Data Collection

Data were acquired from various databases, including Google Scholar, Science Direct, Pub-Med, Scopus, Web of Science, and from library books and theses. The studies ranged from 1991 until January 2012. The key search words included: traditional medicine, medicinal herbs, plant extracts, anti-obesity, weight loss, overweight, botanical remedy, complementary therapy, natural, alternative, phytonutrients, phytochemicals, efficacy, safety, bioactive

Manuscript received May 16, 2012; revised July 16, 2012.

Mahnaz Kazemipoor, Che Wan Jasimah Wan Mohamed Radzi, and Iman Yaze are with the Department of Science & Technology Studies, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia Geoffrey A. Cordell I swith is with Natural Products Inc., Evanston, IL 60203, USA (e-mail: mahnaz@siswa.um.edu.my)

DOI: 10.7763/IJCEA.2012.V3.201

2012 International Conference on Nutrition and Food Sciences
IPCBEE vol. 39 (2012) IACSIT Press, Singapore

Potential of Traditional Medicinal Plants for Treating Obesity: A Review

Mahnaz Kazemipoor1, Che Wan Jasimah Wan Mohamed Radzi2, Geoffrey A. Cordell3, Iman Yaze4

1,2Department of Science & Technology Studies, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
3 Natural Products Inc., Evanston, IL 60203, USA
4Faculty of medicine

Abstract. Obesity is a global health concern associated with high morbidity and mortality. Therapeutic strategies include synthetic drugs and surgery, which may entail high costs and serious complications. Plant-based medicinal agents offer an alternative approach. A review of the studies on accessible botanical sources for the treatment of obesity is provided, which attempts to explain how these medicinal plants act to cause weight loss, and which approach is safer and more efficient. Information was gathered for the period of 1991 to 2012. Five basic mechanisms, including stimulating thermogenesis, lowering lipogenesis, enhancing lipolysis, suppressing appetite, and decreasing the absorption of lipids may be operating. Consumption of standardized medicinal plant extracts may be a safe treatment for obesity. However, some combinations of medicinal plants may result in either lower efficacy or cause unexpected side-effects.

Keywords: Medicinal plants, adipose-tissue differentiation, fat absorption, slimming aids, dietary supplements

1. Introduction

By 2005, obesity had affected 400 million adults [66], and since 1997, WHO has cited obesity as a global epidemic [3, 8]. More than half of the adult population in OECD countries is overweight (body mass index [BMI]≥25 Kg/m2) [10]. According to WHO, obesity is related to cardiovascular diseases, hypertension, diabetes mellitus, gallbladder disease, cancer, endocrine and metabolic disturbances, osteoarthritis, gout, pulmonary diseases, as well as psychological issues, including social bias, prejudice, discrimination, and overeating [65]. Economically, obesity and its health consequences place enormous costs now and for future health care [13, 49, 67]. Being overweight is a cosmetic issue, a major health risk factor [33], and may decrease life expectancy [46]. A proliferation of high-cost, anti-obesity products is in the market [25]. However, they exhibit side effects, such as gastrointestinal and kidney problems [25, 55], and only Orlistat and Sibutramine can be used long-term, in spite of issues regarding weight loss and tolerance [50, 51]. The use of natural remedies for weight loss has increased, based on reliability, safety, and cost compared with synthetic drugs [11] or surgical procedures [12], which may have limitations [40].

1.1. Natural medications

Natural ingredients and medicinal plant preparations may enhance satiety, boost metabolism, and speed up weight loss [34, 43]. Including these foods in the diet may therefore assist slow, individual weight loss. However, doubts about human application remain [58]. Despite the global market for satiety, fat burning, dietary supplements and other weight management remedies, patient awareness of these products is insufficient [15]. Here, a brief review of natural medicinal agents and their anti-obesity potential is presented which could aid patients in selecting a botanical product to develop a healthy body.

+ Corresponding author. Tel.: + (0379675182); fax: + (0379674396).
E-mail address: (jasimah@um.edu.my).

COMPUTER METHODS AND PROGRAMS IN BIOMEDICINE XXX (2014) XXX–XXX

journal homepage: www.intl.elsevierhealth.com/journals/cmpb

Appraisal of adaptive neuro-fuzzy computing technique for estimating anti-obesity properties of a medicinal plant

Mahnaz Kazemipoor[a], Majid Hajifaraji[b,**], Che wan Jasimah Bt wan Mohamed Radzi[a], Shahaboddin Shamshirband[c,e,*], Dalibor Petković[d], Miss Laiha Mat Kiah[c]

[a] Department of Science & Technology Studies, Faculty of Science, University of Malaya, 50603 Kuala Lumpur Malaysia
[b] National Nutrition & Food Technology Research Institute, Faculty of Nutrition & Food Technology, Shahid Beheshti University of Medical Sciences, 1981619573 Tehran, Iran
[c] Department of Computer System and Technology, Faculty of Computer Science and Information Technology, University of Malaya, 50603 Kuala Lumpur, Malaysia
[d] University of Niš, Faculty of Mechanical Engineering, Department for Mechatronics and Control, Aleksandra Medvedeva 14, 18000 Niš, Serbia
[e] Department of Computer Science, Chalous Branch, Islamic Azad University (IAU), 46615-397 Chalous, Mazandaran, Iran

ARTICLE INFO

Article history:
Received 7 August 2014
Received in revised form
16 September 2014
Accepted 8 October 2014

Keywords:
Obesity management
Adaptive neuro-fuzzy system (ANFIS)
Estimation
Carum carvi
Weight loss
Body fat percentage

ABSTRACT

This research examines the precision of an adaptive neuro-fuzzy computing technique in estimating the anti-obesity property of a potent medicinal plant in a clinical dietary intervention. Even though a number of mathematical functions such as SPSS analysis have been proposed for modeling the anti-obesity properties estimation in terms of reduction in body mass index (BMI), body fat percentage, and body weight loss, there are still disadvantages of the models like very demanding in terms of calculation time. Since it is a very crucial problem, in this paper a process was constructed which simulates the anti-obesity activities of caraway (Carum carvi) a traditional medicine on obese women with adaptive neuro-fuzzy inference (ANFIS) method. The ANFIS results are compared with the support vector regression (SVR) results using root-mean-square error (RMSE) and coefficient of determination (R^2). The experimental results show that an improvement in predictive accuracy and capability of generalization can be achieved by the ANFIS approach. The following statistical characteristics are obtained for BMI loss estimation: RMSE = 0.032118 and R^2 = 0.9964 in ANFIS testing and RMSE = 0.47287 and R^2 = 0.361 in SVR testing. For fat loss estimation: RMSE = 0.23787 and R^2 = 0.8599 in ANFIS testing and RMSE = 0.32822 and R^2 = 0.7814 in SVR testing. For weight loss estimation: RMSE = 0.00000035601 and R^2 = 1 in ANFIS testing and RMSE = 0.17192 and R^2 = 0.6607 in SVR testing. Because of that, it can be applied for practical purposes.

* Corresponding author. Tel.: +60 146266763; fax: +60 3 79674396.
** Corresponding author.
E-mail addresses: m39faraji@yahoo.com (M. Hajifaraji), shamshirband@um.edu.my (S. Shamshirband).

http://dx.doi.org/10.1016/j.cmpb.2014.10.006
0169-2607/

Please cite this article in press as: M. Kazemipoor, et al., Appraisal of adaptive neuro-fuzzy computing technique for estimating anti-obesity properties of a medicinal plant, Comput. Methods Programs Biomed. (2014), http://dx.doi.org/10.1016/j.cmpb.2014.10.006

APPENDIX H: APPROVAL OF MEDICAL ETHICS COMMITTEE

UNIVERSITI MALAYA
PUSAT PERUBATAN UM

MEDICAL ETHICS COMMITTEE
UNIVERSITY MALAYA MEDICAL CENTRE
ADDRESS: LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA
TELEPHONE: 03-79493209/2251 FAXIMILE: 03-79494638

NAME OF ETHICS COMMITTEE/IRB: Medical Ethics Committee, University Malaya Medical Centre **ADDRESS:** LEMBAH PANTAI 59100 KUALA LUMPUR	**ETHICS COMMITTEE/IRB REFERENCE NUMBER:** 925.15
PROTOCOL NO: **TITLE:** Resubmission- A study on caraway (Persian cumin) intake as a sustainable dietary practice: Impact on obese and overweight women	
PRINCIPAL INVESTIGATOR: Mrs. Mahnaz Kazemipoor **TELEPHONE:** **KOMTEL:**	**SPONSOR:**

The following item [✓] have been received and reviewed in connection with the above study to be conducted by the above investigator.

[✓] Application Form — Ver date: 07 Jun 12
[✓] Study Protocol — Ver date:
[ ] Investigator's Brochure — Ver date:
[✓] Patient Information Sheet
[✓] Consent Form
[ ] Questionnaire
[✓] Investigator(s) CV's (Mrs. Mahnaz Kazemipoor)

and have been [✓]

[✓] Approved
[ ] Conditionally approved (identify item and specify modification below or in accompanying letter)
[ ] Rejected (identify item and specify reasons below or in accompanying letter)

Comments:

Investigator are required to:

1) *follow instructions, guidelines and requirements of the Medical Ethics Committee.*
2) *report any protocol deviations/violations to Medical Ethics Committee.*
3) *provide annual and closure report to the Medical Ethics Committee.*
4) *comply with International Conference on Harmonization – Guidelines for Good Clinical Practice (ICH-GCP) and Declaration of Helsinki.*
5) *note that Medical Ethics Committee may audit the approved study.*

Date of approval: 20th JUNE 2012

c.c Head
Department of Science & Technology Studies
Faculty of Science, UM

Deputy Dean (Research)
Faculty of Medicine

Secretary
Medical Ethics Committee
University Malaya Medical Centre

.....
PROF. DATUK LOOI LAI MENG
Chairman
Medical Ethics Committee