



UNIVERSITI PUTRA MALAYSIA

**ANTI -ULCER EFFECTS OF Piper betel, Solanum nigrum AND
Zingibercassumunar ON ULCERATION INDUCED BY SELECTED
ULCEROGENS IN RATS**

SHAMIMA ABDUL RAHMAN

FPSK(M) 2005 22

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**By
SHAMIMA ABDUL RAHMAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

April 2005



DEDICATION

“Dedicated especially to my parents (Abdul Rahman Samsudin and Bedah Musooh), brothers (Sallehudin, Islahudin and Hairudin), friends and all individuals who make me possible in completing my study successfully.”



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Associate Proffesor Muhammad Nazrul Hakim Abdullah, PhD

Faculty : Medicine and Health Sciences

Piper betel, *Solanum nigrum* and *Zingiber cassumunar* have been used among Malay community in traditional medicine for curing many diseases. These plants are believed to protect the mucosal lining of the stomach from ulcerogens. This study was conducted to investigate the antiulcerogenic properties of ethanol extracts of these plants. One hundred and forty four Sprague Dawley rats were used in this study. The rats were divided into two major groups, the control and treatment group. There were 6 groups of control rats (normal saline (A1); ethanol (B1); aspirin (C1); acetic acid (D1); indomethacin (E1); and cimetidine (F1)). All control groups received distilled water *ad libitum* 7 days and fasted 24 hours prior forced fed either with normal saline, ethanol, aspirin, acetic acid, indomethacin and cimetidine accordingly. Treated groups were given 2mg / 100g body weight per day of *Piper betel*, *Solanum nigrum* and *Zingiber cassumunar* extract for 7 days, fasted 24 hours prior force fed with either normal saline (Group A2, A3, A4); ethanol (Group B2, B3, B4); aspirin (Group C2, C3, C4); acetic



acid (Group D2, D3, D4); indomethacin (Group E2, E3, E4) or cimetidine (Group F2, F3, F4) accordingly. They were 18 groups all together. They were then fasted again for 24 hours before sacrificed. Macroscopically, the stomachs of rats treated with all plant extracts of all ulcerogen groups (B2, B3, B4; C2, C3, C4; D2, D3, D4; E2, E3, E4; F2, F3, F4) showed a reduced ulcers with significant different ($p < 0.05$) when compared to B1, C1, D1 and E1 control groups. Even though treated groups showed some edema and small ulcers, they gave less lesion score when compared to B1, C1, D1 and E1 control group. Histological findings showed reduced damage and inflammation score without erosion of mucosal layer in groups treated with *Piper betel*, *Solanum nigrum* and *Zingiber cassumunar* when compared to B1, C1, D1 and E1 groups. Even though there was slight epithelial disruptions, minimal inflammatory reactions and presence of red blood cells, the damage and inflammation score were less and significantly reduced ($p < 0.05$) when compared to control groups. This present study suggests that *Piper betel*, *Solanum nigrum* and *Zingiber cassumunar* have some beneficial properties implying that they may have some protective mechanisms to protect stomach from ulcerogens.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN ANTI –ULSER DARI *Piper betel*, *Solanum nigrum* DAN *Zingiber cassumunar* KEATAS ULSER YANG DIRANGSANG OLEH BAHAN-BAHAN PENYEBAB ULSER TERPILIH PADA TIKUS

Oleh

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April 2005

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Piper betel, *Solanum nigrum* dan *Zingiber cassumunar* telah digunakan didalam perubatan tradisional oleh komuniti Melayu untuk mengubati berbagai penyakit. Tumbuh-tumbuhan ini dipercayai dapat memberikan perlindungan kepada lapisan mukosa perut daripada bahan penyebab ulser. Kajian ini dijalankan bagi menyelidiki kesan anti ulser dalam tumbuh-tumbuhan ini. Ekstrak etanol daripada daun tumbuh-tumbuhan ini disediakan dengan menggunakan peralatan ekstrak soxhlet. Seratus empat puluh empat tikus dari jenis Sprague Dawley telah digunakan dalam penyelidikan ini. Didalam kajian ini, tikus-tikus tersebut telah dibahagikan kepada dua kumpulan besar iaitu kumpulan kawalan dan kumpulan rawatan. Terdapat enam kumpulan kawalan (normal saline (A1); etanol (B1); aspirin (C1); asid asetik (D1), indometacin (E1) dan cimetidine (F1)) dalam kajian ini. Kesemua kumpulan tersebut diberikan air suling selama tujuh hari sebelum dibiarkan berpuasa selama 24 jam. Kemudian ia akan diberikan samada normal salin, etanol, aspirin, asid asetik, indometacin atau cimetidine. Kemudian tikus-tikus tersebut dipuaskan sebelum dibunuh. Tikus-tikus dari kumpulan rawatan diberikan ekstrak tumbuh-tumbuhan selama tujuh hari sebanyak 2mg / 100 g per berat badan secara paksa. Ia kemudian

dipuasakan selama 24 jam sebelum diberikan normal saline (Kumpulan A2, A3, A4); ethanol (Kumpulan B2, B3, B4); aspirin (Kumpulan C2, C3, C4); asid asetik (Kumpulan D2, D3, D4); indometacin (Kumpulan E2, E3, E4) atau cimetidine (kumpulan F2, F3, F4). Keseluruhannya terdapat 18 kumpulan dalam kumpulan rawatan ini. Kemudian mereka dipuasakan sebelum dibunuh. Tikus-tikus ini kemudiannya dibedah dan dikeluarkan perutnya untuk diperiksa dan diberikan skor yang bersesuaian. Ia kemudian diproses dan diwarnakan dengan pewarnaan H & E sebelum diperiksa dibawah mikroskop dan diberikan skor kerosakan dan inflamasi. Secara makroskopik, kesemua perut-perut tikus yang diberikan ekstrak tumbuhan dari semua kumpulan penyebab ulser (B2, B3, B4; C2, C3, C4; D2, D3, D4; E2, E3 dan E4) menunjukkan kesan kekurangan ulser yang bererti ($p < 0.05$) jika dibandingkan dengan B1, C1, D1 dan E1 kumpulan-kumpulan rawatan. Walaupun didapati dari kumpulan-kumpulan rawatan tersebut pembengkakan dan ulser kecil, tetapi kurang jika dibandingkan dengan kumpulan kawalan. Penemuan histologi menunjukkan penurunan skor kerosakan dan inflamasi pada lapisan mukosa pada kumpulan yang diberikan ekstrak *Piper betel*, *Solanum nigrum* dan *Zingiber cassumunar* jika dibandingkan dengan kumpulan B1, C1, D1 dan E1. Walaupun terdapat sedikit perubahan pada lapisan epitelia, sedikit sel inflamasi dan kehadiran sel darah merah, tetapi skor kerosakan dan inflamasi menurun dan memberikan kesan signifikan yang bererti ($p < 0.05$) dibandingkan dengan kumpulan kawalan. Kajian ini mencadangkan bahawa *Piper betel*, *Solanum nigrum* dan *Zingiber cassumunar* mempunyai kesan perbaikan dimana mereka dapat memberikan mekanisme pertahanan untuk melindungi lapisan perut tikus daripada ulser.



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I certify that an Examination Committee met on 26th April 2005 to conduct the final examination of Shamima Abdul Rahman on her Master of Science thesis entitled “Anti-ulcer Effects of *Piper betel*, *Solanum nigrum* and *Zingiber cassumunar* on Ulceration Induced by Selected Ulcerogens in Rats” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



SHAMIMA ABDUL RAHMAN

Date: 28/6/05

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LIST OF ABBREVIATIONS/ GLOSSARY OF TERMS

<	Less than
>	More than
AIDS	Acute immunodeficiency syndrome
BW	Body weight
CNS	Central nervous system
DPX	Depex
e.g.	Example
GIT	Gastrointestinal tract
H&E	Hematoxylin and Eosin
H ⁺	Hydrogen ion
H ₂	Histamine ₂
HCl	Hydrochloric acid
hr	Hour
i. e.	In example
IMR	Institute for Medical Research
K-ATPase	Kalium-adenosine triphosphatase
L	Litre
LM	Light Microscope
MARDI	Malaysia Agriculture Research and Development Institute
mg	Milligram
ml	Millilitre
NaCl	Sodium Chloride
NSAIDs	Non-steroidal anti inflammatory drugs
PAF	Platelet activating factor
PG	Prostaglandin
pH	$-\log_{10}[\text{H}^+]$
RBC	Red blood cells
S.E.M	Standard error of mean



CHAPTER 1

INTRODUCTION

1.1 Introduction

The use of plants for the treatment of diseases is a very common practice among the Malay population. This is based upon the assumption that these plants have therapeutic activities without any adverse toxic effects.

Drugs from plants continue to be a big business in the United States of America, with official sales in the year 2000 alone at \$10 billion (Katzung, 1998). More than 200 organizations worldwide are investigating new uses of plant-derived drugs, especially in the fight against AIDS, cancer, diabetes and cardiovascular disease (Liska, 2000). Now, mainstream modern technology is just beginning to re-focus on herbs and natural plant therapies as a primary means to deal with current day health issues (Sook, 1999). Even many drugs commonly used today are herbal in origin (Peter, 1998).

Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world (Basil & Howard, 1995; Nash *et al.*, 1994). Gastric ulcers arise due to various factors (Mc Guigan, 1991) for example, stress, smoking, nutritional deficiencies and ingestion of nonsteroidal-anti inflammatory drugs (Basil & Howard, 1995; Nash *et al.*, 1994). Even though the etiology of gastric ulcers is still debated, it is accepted that



ulcers are caused due to net imbalances in mucosal offensive and defensive factors (Goel & Bhattacharya, 1991) such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermic growth factor) (Salas, 1990).

Ulcer therapy is now mainly focused on limiting the deleterious effects of offensive acid secretion, but the search for new safer alternative drugs has rekindled interest in cytoprotective drugs, which protect the gastric mucosa from damaging agents without influencing acid secretion or neutralizing intragastric acidity (Robert, 1979). Although few drugs like sucralfate and prostaglandin analogues, i.e. misoprostol are recognized as cytoprotective agents (Vergin & Kori-Linder, 1990), many natural drugs have been reported to possess this activity too (Goel *et al.*, 1985; Rao *et al.*, 2000 & Sairam *et al.*, 2001).

Herbal medicine is uniquely suited for the treatment of illness of digestive system. Throughout the evolutionary process, our food has been our medicine, ensuring that the distinctive healing properties of herbs have a direct effect on the lining of the alimentary canal. Not only will there be therapeutic effects due to the metabolism and absorption of the whole range of constituents present in the plant, but there will also be some direct action on the tissue of the gut through contact (Hoffman, 1990).

Several plants have been studied worldwide for their efficacy against gastric ulcers, such as *Pyrenacantha standii* (Aguwa & Mittal, 1981; Aguwa & Okunji, 1986),



Cinnamomum cassia (Akira *et al.*, 1986), *Musa* species (Best *et al.*, 1984), *Aloe vera* (Parmar *et al.*, 1986), *Laurus nobilis* (Afifi *et al.*, 1997), *Emblica officinalis* (Sairam *et al.*, 2002) *Sapindus saponaria* L (Adriana *et al.*, 2002), *Maytenus ilicifolia* (Carmen *et al.*, 2002), *Camellia sinensis* (Maity *et al.*, 1995) and *Sophora subprostata* (Konturek *et al.*, 1986).

Piper betel, *Solanum nigrum* and *Zingiber cassumunar* have long been used in the traditional medicine in Malaysia (Abd. Samad, 1985). In Malay community, these plants can be easily found because these plants are easy to grow (Abd. Rahman, 1998). They are known to cure many diseases and famously used by traditional practitioner. These plants were believed by traditional practitioner as anti ulcer agents, but there is no laboratory finding to prove this claim.

Piper betel or “sireh” is an important commercial herb where it is popularly used in the ‘betel chew’ by the Indians. Commonly, it is called as “betelvine” in English, “tambuli” in India, “tambulai” in Arabic and “sireh” in Indonesia and Malaysia (Weiss, 1997). In Malaysia, they are popularly used fresh and grown in home gardens (Duke, 2001). All parts of the plants are used in traditional Indian and Greek medicine. Their medicinals use range from treatments of ulcers, respiratory ailment to afterbirth tonics (Duke, 2001)

Solanum nigrum Linn or Black Nightshade is the plant of the family Solanaceae, known as “Terung Meranti” in Malay community or Daun Ranti (Abd. Rahman, 1998).



In Malay community, it is believed to have therapeutic effects and used traditionally to cure cough, bronchitis, wound, ulceration, vomiting and swelling (Abd. Samad, 1985).

Zingiber cassumunar is also known as Bungelai, Bongelai, Bolai, Kunyit bolai, Bulai, Boleh in Java, Bengle, Bangle, Kunyit bolai; in Sundanese (Burkill, 1996), known as Kunyit Bolai or Bonglai in Malaysia (Mohd. Said *et al.*, 2000) and also called “plai” in Thailand (Weiss, 1997). *Z. cassumunar* are used for aches and pains, inflammations, joint problems, muscle spasms, sprains and strains, torn muscles and ligaments asthma, catarrh, chronic colds, colic, constipation, diarrhea, fevers, flatulence, heartburn, immune problems, influenza, nausea and respiratory problems (Burkill, 1996).

1.2 Objectives

This present study was carried out based on the traditional use of these plants to cure ulcers and the need of natural plants that can alternatively relieve the inflammation process in gastrointestinal tract. Even though in Malay community these plants were believed to cure many diseases include gastric ulcer, it is in need to prove this believed in proper laboratory findings.