UNIVERSITI PUTRA MALAYSIA

ANTIULCER PROPERTIES OF ESSENTIAL OIL AND POLYPEPTIDE K ISOLATED FROM Momordica charantia L. SEEDS

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By

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ANTIULCER PROPERTIES OF ESSENTIAL OIL AND POLYPEPTIDE K ISOLATED FROM *Momordica Charantia* L. SEEDS

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November 2015

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Momordica charantia L. or bitter gourd, a Cucurbitaceae family plant is a plant native to the semi-tropical climate of Thailand, Asia, India and Africa and has been traditionally used as a folk remedy and best known for its anti-diabetic, anti-inflammatory, anti-microbial, anti-ulcer and antihelmintic properties. The aims of this study were to investigate the anti-ulcerogenic activities of *Momordica charantia L.* (MC) essential oil and polypeptide k on various rats model. The anti-ulcerogenic effects of MCEO and polypeptide k were studied against HCl/Ethanol and Indomethacin-induced ulcer in rats. *Spraque Dawley* rats were given treatment orally for 7 days consecutively. In pre-treatment for MCEO, total length for HCl/EtOH is significantly longer than indomethacin Generally, for negative control, total length is 48.2±19.9. Supplementation with 10 MCEO, it reduced to 19.3±13.1 and similar to Rantidine 100 mg/kg. When the dose was increased, the total length was decreased (19.3±13.1 to 6.2±6.2). Rantidine 100 mg/kg as reference drug reduced the length about half of the negative control group. Furthermore, polypeptide k showed significantly longer (ulcer length) in HCl/EtOH than indomethacin. Generally, for negative control, total length is 43.0±14.1. Supplementation with 10 PPK, it reduced to 29.3±18.6 and slightly similar to Rantidine 100 mg/kg. When the dose was increased, the total length was decreased (29.3±18.6 to 13.6±9.5). Rantidine 100 mg/kg as reference drug reduced the length about half of the negative control group (22.1±13.2). This has been supported by findings from pylorus-ligated model in rats. Pre-treatment with MCEO at 10 mg/kg, 50 mg/kg and 100 mg/kg failed to increase the volume of gastric acid secretion when compared to control group. However, it significantly elevates the pH but not decrease the total acidity. Basically, MCEO managed to preserve the gastric wall by significantly increased the gastric wall mucus content. Polypeptide K (PPK) on the other hand, exerted a significant reduction in the total ulcer area (mm^2) similarly with Ranitidine and successfully preserve the gastric

wall by significantly increase the gastric wall mucus. However, it did not elevate the pH nor decrease the total acidity. As a conclusion, MCEO and polypeptide k possesses anti-ulcer effects in various ulcer models of rats.

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

POTENSI ANTI-ULSER OLEH MINYAK PATI DAN POLIPEPTIDA K PENGASINGAN DARI BIJI *Momordica charantia* L.

Oleh

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Momordica charantia L . atau peria, berasal dari keluarga Cucurbitaceae merupakan tumbuhan asli untuk iklim separa tropika Thailand, India, Asia dan Afrika dan telah secara tradisional digunakan sebagai ubat rakyat seperti anti-diabetes, anti-radang, anti-mikrob, anti ulser dan antihelmintic sifatnya. Matlamat kajian ini adalah untuk menyiasat aktiviti-aktiviti anti ulcerogenic minyak pati *Momordica charantia L.*(MC) dan polipeptida k ke atas pelbagai model tikus. Kesan anti-ulser MCEO dan polypeptida k telah dikaji terhadap aruhan HCl/etanol dan Indomethacin pada tikus. Tikus *Spraque Dawley* telah diberi rawatan secara oral selama 7 hari berturut-turut. Dalam pra-rawatan untuk MCEO, jumlah panjang ulser untuk HCl / EtOH adalah jauh lebih panjang daripada indomethacin. Secara umumnya, untuk kawalan negatif, jumlah panjang adalah 48.2 ± 19.9. Pada dos 10 MCEO, ia berkurang kepada 19.3 ± 13.1 dan sama dengan Ranitidine 100 mg / kg. Apabila dos meningkat, jumlah panjang telah menurun (19.3 ± 13,1-6,2 ± 6.2). Ranitidine 100 mg / kg sebagai ubat rujukan dikurangkan panjang kira-kira separuh daripada kumpulan kawalan negatif. Tambahan pula, polipeptida k menunjukkan panjang ulser yang ketara dalam HCl / EtOH daripada indomethacin. Secara umumnya, untuk kawalan negatif, jumlah panjang adalah 43.0 ± 14.1. Pada dos 10 mg/kg PPK, ia dikurangkan kepada 29.3 ± 18.6 dan sedikit sama dengan Rantidine 100 kg mg /. Apabila dos meningkat, jumlah panjang telah menurun (29.3 ± 18,6-13,6 ± 9.5). Rantidine 100 mg / kg sebagai ubat rujukan dikurangkan panjang kira-kira separuh daripada kumpulan kawalan negatif (22.1 ± 13.2). Ini telah disokong oleh penemuan-penemuan daripada model pylorus-ligated pada tikus. Pra rawatan dengan MCEO 10 mg/kg, 50 mg/kg dan 100 mg/kg gagal meningkatkan rembesan asid gastrik jika dibandingkan dengan kumpulan kawalan. Walau bagaimanapun, ia menaikkan pH yang ketara tetapi tidak mengurangkan jumlah keasidan. Pada dasarnya, MCEO dapat mengekalkan dinding gastrik dengan meningkatkan kandungan mukus dinding gastrik. Polipeptida K (PPK) di sisi lain, memberikan pengurangan yang ketara di kawasan jumlah ulser (mm^2)

sama seperti dengan kumpulan Ranitidine dan berjaya mengekalkan dinding gastrik dengan meningkatkan mukus dinding gastrik secara signifikan. Walau bagaimanapun, ia tidak pula meningkatkan pH atau mengurangkan jumlah keasidan. Sebagai kesimpulannya, MCEO dan polipeptida k mempunyai kesan anti-ulser dalam pelbagai model ulser tikus.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

MC	*Momordica charantia*
MCEO	*Momordica charantia* essential oil
PPK	Polypeptide K
NSAIDS	Non-steroidal Anti-Inflammatory Drugs
ANOVA	Analysis of Variance
S.E.M	Standard Error of Mean
COX	Cyclooxygenase enzyme
cm	centimeters
ml	mililitres
g	gram
mg	milligram
kg	kilogram
nm	nanometer
mm^2	millimeter square
HCl	hydrochloric acid
EtOH	ethanol
PUD	Peptic Ulcer Disease
H&E	Hematoxylin and eosin
NaOH	Sodium hydroxide
$MgCl_2$	Magnesium chloride
Cl^-	Chloride ion
K^+	Potassium ion
H^+	Hydrogen ion
HCO_3^-	Bicarbonate ion

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Gastric ulcer is defined as disruption or erosion of the mucosal integrity of the stomach which extends through the muscularis mucosa into submucosa or deeper (Singh, et.al., 2008). It also demonstrates a local defect or excavation at the mucosal surface due to active inflammation (Das, et. al., 2008). Gastric ulcer is a very common global problem and gastrointestinal disorder today as it is caused by various factors. As well known, ulcer disease results from an imbalance between aggressive and defensive factors (Jainu, et.al., 2006). Acid, pepsin, *Helicobacter pylori* and bile salts are the major aggressive factors while defensive factors mainly involve mucus-bicarbonate secretion and prostaglandins (Hoogerwerf and Pasricha, 2001). Common causes involved in the development of gastric ulceration are *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs (NSAIDs).

Helicobacter pylori, a Gram-negative bacteria weakens the protective coating of the stomach and first part of the intestine thus allows damaging digestive juices to eat away at the sensitive lining below. Frequent ingestion of NSAIDs, stress, smoking and nutritional deficiencies also will increase the gastric ulcer incidences (Belaiche, et al., 2002). Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are commonly used as pain relievers, but prolonged used of NSAIDs will demonstrate the blockage of prostaglandins, release a substance in the stomach which help to maintain blood flow and protect the mucosal area from injury.

There are many plants with ethnopharmacological background that have been used in traditional medicine known to possess anti-ulcer properties. These include *Bauhinia purpurea* (Zakaria, et. al., 2011), *Alchornea castaneaefolia* (Hiruma-Lima, et. al., 2006), *Solanum nigrum* (Jainu, et. al., 2006), *Kaempferia parviflora* (Rujjanawate, 2005), *Utleria salicifolia* (Rao, et. al., 2004) and many more.

Momordica charantia L., a Cucurbitaceae family plant is a plant native to the semi-tropical climate of Thailand, India, Asia and Africa has been traditionally used as a folk remedy and best known for its anti-diabetic (Satishsekar and Subramanian, 2005a), antifungal (Schmourlo, et. al., 2005), antiulcer (Samsul Alam, 2009), antioxidant effects (Sathishsekar and Subramanian, 2005b), antihyperlipidemic (Chen and Li, 2005), antimutagenicity (Singh et. al., 1998) and antiviral (Jjiratchariyakul, et. al., 2001) properties. The oil from the seeds of Momordica charantia L. useful as anti-inflammatory, anti-arthritic, vasculodilatory and wound healing agent (Khanna, 2005). In addition, Polypeptide k, isolated from seeds of MC has anti-diabetic properties which is more potent than polypeptide-p and helps in preventing diabetes (Kanna, 2004). However, no scientific data is available on the anti-ulcer properties of

MC oil and polypeptide k Therefore, this present study was aimed to investigate the antiulcer properties of essential oil and polypeptide k isolated from *Momordica charantia* L. seeds.

1.2 Problem Statement

Gastric hyperacidity and gastrodoudenal ulcer is a very common global problem and causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Recent data from Malaysia suggest that prevalence of duodenal ulcer and gastric ulcer is 9.5% and 9.4% respectively (Goh, et al, 2009). There is a report stated that the prevalence of peptic ulcer disease in the Western countries has been declined as well as in the Asia-Pacific region (El-Serag and Sonnenberg, 1998). However, recent study showed that there was an increase of the diagnosis of erosive gastritis in Turkey (Erkan Caglar, et. al, 2014). *Helicobacter pylori (H. pylori),* is a bacterium that infects the lining of the stomach and causes chronic inflammation and ulcers is believed to be one of the causes of gastric cancer. In Malaysia, gastric cancer is the ninth most common cancer in the entire general population and the eight most common cancers in males and tenth in females in Malaysia (National Cancer Registry, Malaysia, 2007). The incidence of gastric cancer increases with age and slightly higher in males compared to females and Chinese were found to have higher incidence rate compared to Malay and Indian (National Cancer Registry, Malaysia, 2007).

The conventional drugs used in the treatment of gastric ulcer include proton pump inhibitors (omeprazole, lansoprazole), histamine H2 receptor antagonists (ranitidine, famotidine), antacids and anticholinergics. However, there are reports stating that most of these drugs produce several adverse reactions (Brunton, 1998). In addition, increase in NSAIDs use in recent years is believed to be the reason for the increase of gastric ulcer cases (Erkan Caglar, et. al, 2014). Thus, there is a need for more effective and safe anti-ulcer agents. It is believed that most of the herbal medicine will reduce the offensive factors and proved to be safe, clinically effective, better patient tolerance, relatively less expensive and globally competitive (Goel and Sairam, 2002). Plant extracts, have been proven to produce promising results in treating the gastric ulcers (Jainu, et.al., 2006). Hence, more traditional medicine plants are needed to be explored in searching the best treatment of ulcer.

1.3 Significance of Study

Although gastric ulcer may not be a major health problems in Malaysia, but it is important to have a precaution and treatment steps towards it. The findings of this study are important to reduce the gastric hyperacidity and gastrodoudenal ulcer cases in this country by using plant extract as an alternative to conventional drugs. On the other side, this study will help in treating the gastrodoudenal ulcers therefore developed other options for general practitioners as well as the patients. In addition, natural products used in medical, may reduce the cost and reduce the mortality cases

among patients. With all the information obtained, this study could improve in healthcare services thus contribute to help the community for the better living.

1.4 Research Objectives

General objective:

- To investigate the anti-ulcerogenic activities of essential oil and polypeptide k extracted from *Momordica charantia L.* using rats model.

Specific objectives:

- To compare the macroscopic and microscopic effect of *Momordica charantia* seed oil and polypeptide k in ulcer-induced rats by HCl/Ethanol and Indomethacin.
- To evaluate the mechanism of *Momordica charantia* essential oil and polypeptide k by measuring gastric acid secretion in pylorous ligated rats.

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