UNIVERSITI PUTRA MALAYSIA

ANTIULCEROGENIC EFFECT OF Momordica charantia ON HCL/ETHANOL INDUCED RATS

BY

MOHD NASIER BIN KAMALDIN

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ABSTRACT

Gastric ulcer is a common disorder where discontinuity in the gastric mucosa is observed. It is caused by many factors like stress, drugs, alcohol and is reported to be due to an imbalance between offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion and cell shedding. The conventional drugs used in the treatment of gastric ulcer include histamine (H2) receptor antagonists, proton pump inhibitors, antacids and anticholinergics. However, most of these drugs contribute various undesirable side effects and drug interactions. Use of natural drugs in gastric ulcers is well documented. Most of these drugs augment the mucosal defensive factors, which are thought to be important for protection of gastric mucosa. One such plant is *Momordica charantia*, used in traditional medicine against ulcer. It has been frequently used in folk medicine for rapid healing of cutaneous lesions and peptic ulcer in Asia and Africa. Therefore this study has designed to evaluate the effect of *Momordica charantia* seed oil on HCl/ethanol induced ulcer in experimental rats. Antiulcer assay performed by using HCl/ethanol combination method. Animal pretreated for 7 days with distilled water (negative control), ranitidine (positive control – 100 mg/kg) and with bitter gourd seed oil in various doses (100%, 50% and 10%) according to their to their body weight (10 ml/kg) and respective to the groups. On day 7, after one hour of pretreatment, animals fed orally with HCl/ethanol and one hour later, animals were sacrificed and proceed to macroscopic and microscopic assessment. From this study, *M. charantia* possess a significant anti-ulcer activity in HCl/ethanol – induced rats. The highest doses of treatment, XO-100%, showed the most effective and significant ($p < 0.005$) result followed by XO-50% and XO-10%. The XO-100% group show maximum inhibition of ulcer length and ulcer lesion by 90.95% and 68.96% respectively. XO-50% group inhibited ulcer length by 74.95% and ulcer lesion by 44.82%. The XO-10% is less

effective in inhibition of ulcer length and ulcer lesion by 61.30% and 24.12% respectively. In future, this project could be able to explore a new concept of using bitter gourd seed oil as an anti ulcer agent instead of other remedy from traditional plant extract or existing therapy. This new agent not only reduces the problem associated with peptic ulcer but also provide therapeutic option for treating other type of ulcer.

KESAN ANTIULSER BAGI *Momordica charantia* TERHADAP TIKUS YANG DIRANGSANG OLEH HCl/ETHANOL

MOHD NASIER BIN KAMALDIN

ABSTRAK

Ulser gastrik ialah penyakit biasa dimana ketidaknormalan di gastrik mucosa dapat dilihat. Ia disebabkan oleh banyak factor antaranya ialah tekanan, ubat, alkohol and dilaporkan disebabkan oleh ketidakseimbangan antara rembesan asid-pepsin dan faktor pertahanan seperti rembesan mucin dan lindungan sel. Ubat yg digunakan untuk merawat ulser gastrik seperti histamine (H2) receptor antagonists, proton pump inhibitors, antacids dan anticholinergics. Tetapi kebanyakan ubat ini akan menyebabkan kesan sampingan dan interaksi ubat. Kegunaan ubat tradisional unuk merawat ulser gastrik telah banyak didokumenkan. Kebanyakan ubat ini menambah factor pertahanan mucosa yang penting untuk melindungi gastrik mucosa. Salah satu tumbuhan ialah *Momordica charantia,*digunakn sebagai perubatan tradisional terhadap ulser. Ianya kerap digunakan di Asia dan Africa sebagai rawatan segera untuk kecedaran kulit dan ulser peptic. Oleh sebab itu, kajian direka untuk melihat kesan minyak benih *Momordica charantia* terhadap tikus eksperimen yang dirangsang oleh HCl/etanol. Antiulser assay dijalankan menggunakan kaedah gabungan HCl/etanol. Haiwan dirawat selama 7 hari mengunakan dengan air suling (kawalan negatif), ranitidine (kawalan positif – 100 mg/kg) dan dengan minyak benih peria yang berlainan sukatan (100%, 50% and 10%) mengikut berat badan haiwan (10 ml/kg) dan kumpulan masing-masing. Pada hari ke 7, selepas satu jam memberi rawatan, haiwan tersebut diberi HCl/etanol dan selepas satu jam berikutnya, haiwan tersebut dikorbankan untuk membuat penilaian makroskopik dan mikroskopik. Daripada kajian ini, didapati bahawa *M. charantia* mempunyai aktiviti antiulser yang signifikasi terhadap tikus yang dirangsang HCl/etanol. Dos tertinggi rawatan iaitu XO-100% menunjukan keputusan yang paling efektif dan signifikan ($p <$ 0.005) diikuti dengan dos XO-50% and XO-10%. Kumpulan XO-100% menunjukan perencatan maksimum terhadap panjang ulser dan ulser lesion sebanyak 90.95% dan 68.96% masing-masing. Kumpulan XO-50%, merencatkan panjang ulcer sebanyak 74.95% dan ulser lesion sebanyak 44.82%. Kumpulan XO-10% menunjukan kesan perencatan panjang ulcer dan ulser lesion yg kurang efektif iaitu sebanyak 61.30% and 24.12% masing-masing. Pada masa akan dating, diharap agar projek ini dapat menerokai

konsep baru dalam penggunaan minyak benih peria sebagai agen anti ulser. Agen baru ini juga diharap bukan sahaja merawat ulser peptic malah dapat memberi pilihan rawatan untuk pelbagai jenis ulser yang lain.

ACKNOWLEDGEMENT

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First and foremost, I would hereby emphasize greatest gratitude and appreciation to my supervisor, Associate Professor Dr Muhd Nadzrul Hakim, for his never ending guidance, encouragement, advice, constructive comments and his willingness to help throughout the whole project. It was a great experience for me having this opportunity to do this study under his supervision. To Malarvili Selvaraja, for his kindness in giving advises on experiment being done and willing to share his experience with me.

On the other hand, I would like to express my heartfelt gratitude to my entire course mates for their infinite encouragement, aid and support. Special thank to Siti Safiah for her kind and helpful assistance to make this project such a success and memorable. And to all lecture and staff of the Department of Biomedical Sciences, Faculty Medicine and Health Sciences, University Putra Malaysia, I would like to thanks

all of you for giving me so much knowledge and support throughout my 4 years time here.

Last but not least, I would like to express my heartiest gratitude to my beloved parents and my family for moral and financial support during completing this study. Thanks for their love, care, support, inspiration and constant encouragement throughout the period of the study. Thank you.

ANTIULCEROGENIC EFFECT OF *Momordica charantia* ON HCl/ETHANOL INDUCED RATS

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ABSTRACT

Introduction: Gastric ulcer is a common disorder where discontinuity in the gastric mucosa is observed. It is caused by many factors like stress, drugs, alcohol and is reported to be due to an imbalance between offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion and cell shedding. *Momordica charantia*, used in traditional medicine against ulcer. It has been frequently used in folk medicine for rapid healing of cutaneous lesions and peptic ulcer in Asia and Africa. Therefore this study has designed to evaluate the effect of *M. charantia* seed oil on HCl/ethanol induced ulcer in experimental rats. **Methodology:** Antiulcer assay performed by using HCl/ethanol combination method. Animal pretreated for 7 days with distilled water (negative control), ranitidine (positive control – 100 mg/kg) and with bitter gourd seed oil in various doses (100%, 50% and 10%) according to their to their body weight (10 ml/kg) and respective to the groups. On day 7, after one hour of pretreatment, animals fed orally with HCl/ethanol and one hour later, animals were sacrificed and proceed to macroscopic and microscopic assessment. **Result:** From this study, *M. charantia* possess a significant anti-ulcer activity in HCl/ethanol – induced rats. The highest doses of treatment, XO-100%, showed the most effective and significant ($p < 0.005$) result followed by XO-50% and XO-10%. The XO-100% group show maximum inhibition of ulcer length and ulcer lesion by 90.95% and 68.96% respectively. XO-50% group inhibited ulcer length by 74.95% and ulcer lesion by 44.82%. The XO-10% is less effective in inhibition of ulcer length and ulcer lesion by 61.30% and 24.12% respectively. **Conclusion:** In conclusion, essential oil of *M. charantia* essential oil significantly inhibited ulcer lesion and ulcer length induce by HCl/ethanol. We believed that *M. charantia* possessed the mucoproctective and cytoprotective effect in strengthening the defensive mechanism gastric mucosal since it significantly reduced the gastric lesion area induced by HCl/ethanol.

Keywords: *Momordica charantia*, HCl/ethanol, ranitidine, mucoproctective, cytoprotective

INTRODUCTION

Gastric ulcer is an ulcer that breach and craterlike like lesion in the mucosa of the stomach that extend through the submucosa into muscularis mucosa or deeper (Kumar *et.al,* 2003). It is also a local defect at excavation of the mucosal surface of the stomach and is occupied by the formation of inflammatory necrotic tissue. Ulcer disease results from an imbalance between aggressive factors and the ability of the gastroduodenal mucosal to protect and heal itself. Acid and pepsin secreted by the stomach are key 'aggressive elements' and are generally required for ulcers to develop (Hong, 2002).

One such plant is *Momordica Charantia*, used in traditional medicine against ulcer. The *M. Charantia* or also known as bitter gourd has excellent medicinal virtues. The fruits of *Momordica charantia* are reported to possess wide range of pharmacological activities such as hypoglycaemic (Zheng et al., 2005), antidiabetic (Sathishsekar and Subramanian, 2005a), antifungal (Schmourlo et al., 2005), inhibition of p-glycoproteins (Limtrakul et al., 2004), antihyperlipidemic (Chen and Li, 2005) and antioxidant effects (Sathishsekar and Subramanian, 2005b).

In this study, we are going to investigate anti ulcerogenic effect of *Momordica charantia* on HCl/ethanol induced rats. The reason for selecting *Momordica charantia* in this present study is because of its strong ethnopharmacological properties. This study was done in order to produce better and safer alternative for the treatment of gastric ulcer with implementation in food medicines especially as a supplement product. This study also believes to lead us in using the active compound of *M. charantia* to treat many diseases and would give beneficial effect to our health.

MATERIALS AND METHODS

Production of *M. charantia* essential oil

Essential oil was obtained from the dried seeds by hydrodistillation using the Clevenger-type apparatus. Air-dried seeds of M. charantia were ground and subjected to hydrodistillation for 3 hour by use of a Clevenger type apparatus. The essential oil was isolated and dried over anhydrous sodium sulfate.

Experimental animals

Weaned male Sprague–Dawley rats aged 5-6 weeks and weighing 150-200g used in the study. The animals were maintained in standard laboratory conditions (temperature 25 ± 2 ºC). The animals were fed on standard pellet diet. Food was withdrawn 24 h before induction of ulcer to the animals, but the animals were allowed free access to water.

Experimental design

Animal pretreated for 7 days with distilled water(negative control) and ranitidine (positive control – 100 mg/kg) and with *M. charantia* essential oil in various doses (100%, 50% and 10%) according to their to their body weight (10 ml/kg) and respective to the groups. On day 7, after one hour of pretreatment, all groups of animals except for normal group fed orally with HCl/ethanol. Rats were deprived of food but allowed free access of water 12 hour before HCl/ethanol administration. One hour after induce ulcer, animals were sacrificed and the stomach was removed, opened along the greater curvature and sum of length of lesions (mm) was calculated and expressed as lesion index.

HCl/EtOH-induced gastric ulcers

The *M. charantia* essential oil was administered orally to 24h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml EtOH + 1.7 ml HCl + 38.3 mlH2O) p.o. (Mizui and Doteuchi, 1988). The animals were sacrificed and examined for gastric ulcers 60 min later.

Histopathological studies

For histological examination, the stomach tissues involved many processes including grossing, processing, embedding (blocking), trimming, sectioning, fishing, staining and mounting. In this study, kidney tissue was stained with haematoxylin and eosin (H and E) staining in order to examine the histopathological changes.

Statistical analysis

Data was expressed as mean ± S.E.M. the results of ulcer scoring and scoring data of histopathology study was analyzed with non parametric statistical analysis by Kruskal-Wallis test, followed by Man-Whitney U test for comparison within the group. For the ulcer index data was analyzed with one way ANOVA, followed by Dunnets multiple comparison test post-hoc comparison of group means. For all tests, effect of probability of $p<0.05$ were considered significant.

RESULT

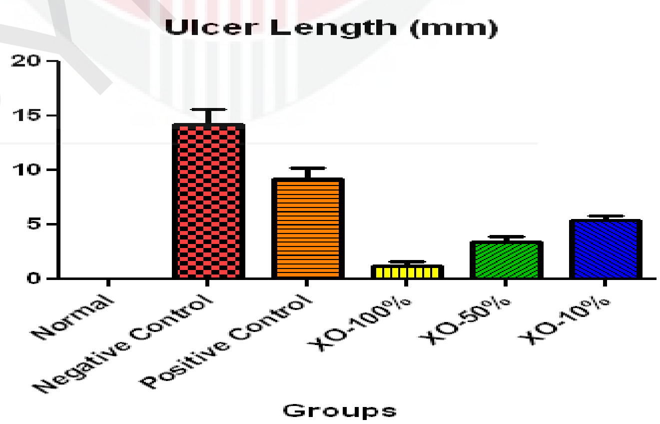

Table 4.1

Effect of *Momordica charantia* L. seed essential oil on ulcer length induced by HCl/EtOH induced ulcer

All values are mean±SEM, $n = 6$.
* $p < 0.05$ when compared to negative control
** $p < 0.01$ when compared to negative control
*** $p < 0.005$ when compared to negative control

Table 4.1, the *Momordica charantia* seed essential oil showed a dose dependent and significant anti-ulcerogenic activity in reducing ulcer length in stomach of HCl/ethanol animal induced model. Pretreatment with XO-100%, showed the most effective result in reducing ulcer length followed by XO-50%, XO-10% and positive control (ranitidine) respectively. XO-100% group significantly reduced ulcer length from 14.17±1.400mm^2 to 1.183±0.4254mm^2 ($p < 0.005$). XO-50% reduced ulcer length from 14.17±1.400mm^2 to 3.450±0.4877mm2 ($p < 0.005$). XO-10% reduced ulcer length from 14.17±1.400mm^2 to 5.383±0.3842mm2 ($p < 0.005$). Ranitidine reduced ulcer length from 14.17±1.400mm^2 to 9.167±1.078mm2 ($p < 0.005$).

Group	Ulcer length (mean±SEM)	Ulcer length inhibition (%)
Negative control	14.17±1.400	-
Positive control (Ranitidine)	9.167±1.078**	34.63
XO-100%	1.183±0.4254***	90.95
XO-50%	3.450±0.4877***	74.95
XO-10%	5.383±0.3842***	61.30

Group	Ulcer lesion (mean±SEM)	Ulcer lesion inhibition (%)

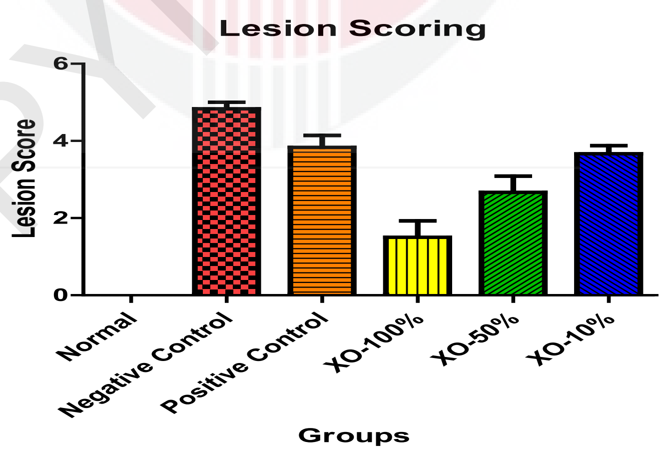

Table 4.2
Effect of *Momordica charantia* L. seed essential oil on ulcer lesion induced by HCl/EtOH induced ulcer

Negative control	4.833±0.1667	-
Positive control (Ranitidine)	3.833±0.3073	20.69
XO-100%	1.500±0.4282***	68.96
XO-50%	2.667±0.4216***	44.82
XO-10%	3.667±0.2108	24.12

All values are mean±SEM, $n = 6$.
* $p < 0.05$ when compared to negative control
** $p < 0.01$ when compared to negative control
*** $p < 0.005$ when compared to negative control

Table 4.2, the *Momordica charantia* seed essential oil showed a dose dependent and significant anti-ulcerogenic activity in reducing ulcer lesion in stomach of HCl/ethanol animal induced model. Pretreatment with XO-100%, showed the most effective result in reducing ulcer leesion followed by XO-50%, XO-10% and positive control (ranitidine) respectively. XO-100% group significantly reduced ulcer lesion from 4.833±0.166mm^2 to 1.500±0.4282mm^2 ($p < 0.005$). XO-50% reduced ulcer lesion from 4.833±0.166mm^2 to 2.667±0.4216mm^2 ($p < 0.005$). XO-10% reduced ulcer lesion from 4.833±0.166mm^2 to 3.667±0.2108mm^2. Ranitidine reduced ulcer lesion from 4.833±0.166mm^2 to 3.833±0.3073mm^2.

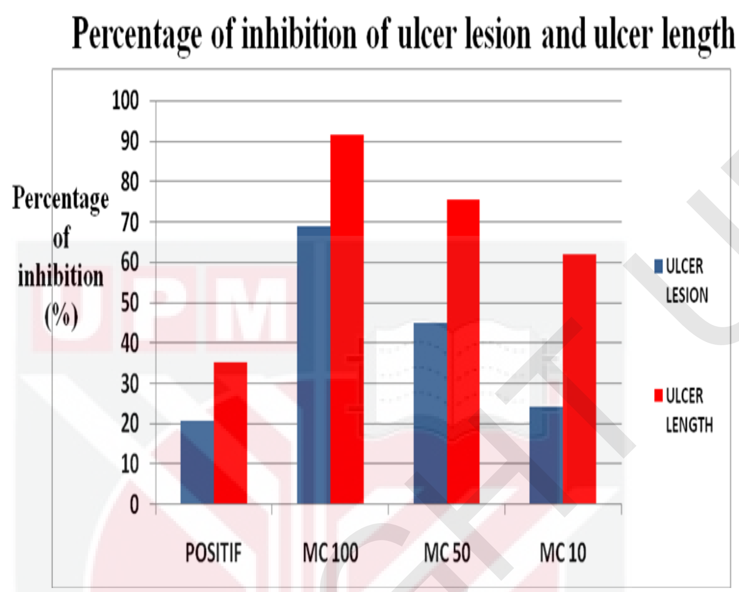

Figure 4.3
Effect of *Momordica charantia* L. seed essential oil on percentage of ulcer lesion and ulcer length induced by HCl/EtOH induced ulcer

As shown in Figure 4.3, the essential oil of M. charantia fruits showed a dose dependent and significant anti-ulcerogenic activity. At higher dose, 100% concentration of M. charantia seed essential oil, it showed maximum inhibition of ulcer length with the percentage of 90.95% and ulcer lesion with percentage of 68.96%. At 50%, it inhibited ulcer length by 74.95% and ulcer lesion by 44.82%. At 10%, it showed lowest inhibition of ulcer length by 61.30% and ulcer lesion by 24.12% if compared to previous group of treatment. Positive group inhibited ulcer lesion by 20.69% and ulcer length by 34.63%.

DISCUSSION

Momordica charantia is known to posses various therapeutic properties and one of the medicinal properties is antiulcerogenic effect from its fruits (Gurbuz, 1999: Yesilada, 1998). However, none has reported on its seed part yet. This also the first study of antiulcerogenic activity used essential oil. Most of other study that involves *M. charantia* used extraction method. The *M. charantia* essential oil at different doses (100%, 50% and 10%) was investigated in HCl/ethanol induced ulcer models.

HCl/ethanol combination which is a necrotizing agent has been used in the present study to induce ulcer in rat's stomach.

M. charantia seed essential oil pretreatment at three different doses (100%, 50% and 10%) was found to offer the gastric mucosal protection, when it exhibited a statistically significant protection against ulcerations induced by HCl/EtOH. Furthermore, the protection effect is exhibited in dose dependant manner. At higher dose, 100% concentration of *M. charantia* seed essential oil has significantly reduced ulcer lesion and ulcer length. For 100% pretreated dose of *M. charantia* essential oil, it showed maximum inhibition of ulcer length with the percentage of 90.95% and reduced ulcer length from 14.17±1.400mm^2 to 1.183±0.4254mm^2 (p < 0.005). It also showed maximum inhibition of ulcer lesion with percentage of 68.96% and reduced ulcer lesion from 4.833±0.166mm^2 to 1.500±0.4282mm^2 (p < 0.005).

The second effective dose for this pretreatment study would be 50% *M. charantia* essential oil group. It showed inhibition of ulcer length with the percentage of 74.95% and reduced ulcer length from 14.17±1.400mm^2 to 3.450±0.4877mm2 (p < 0.005). It also inhibited ulcer lesion with the percentage of 44.82% and reduced ulcer lesion from 4.833±0.166mm^2 to 2.667±0.4216mm^2 (p < 0.005). Pretreatment with 10% of *M. charantia* essential oil showed less effective in reduced ulcer length and ulcer lesion compared to 50% and 100% pretreatment group. The 10% essential oil showed inhibition of ulcer length with the percentage of 61.30% and reduced ulcer length from 14.17±1.400mm^2 to 5.383±0.3842mm2 (p < 0.005). It also inhibited ulcer lesion with the percentage of 24.12% and reduced ulcer lesion from 4.833±0.166mm^2 to 3.667±0.2108mm^2. Treatment with this essential oil showed better result when compared with positive and negative control.

The effectiveness of *Momordica charantia* essential oil in HCl/EtOH induced ulcer may be due to antioxidant activity of *Momordica charantia* that has strong scavenger of superoxide and hydroxyl radicals (Sreejayan, 1991). There are studies that suggested that active oxygen species may be involved in the pathogenesis of gastric mucosal injuries (Szelenyi and Brune, 1988). Radical scavengers were shown to have a protective effect against the gastric mucosal injuries induced by active oxygen species (Oka *et al*., 1991). An in vivo ulcer model for the assessment of effects of antioxidant compounds on the prevention from gastric injuries has been done (Oka *et al*. 1990). *Momordica charantia* was reported in some journal to contain a number of flavonoids, alkaloids and many other active chemical constituents (Ross, 1999). Flavonoids present in different plants are known to reduce gastric ulcer formation (Suzuki et al., 1998); it is assumed that flavanoids may contribute at least in part to the antiulcer effect of *Momordica charantia*. Steroids such as beta sitosterol are known to reduce the development of gastric ulcers (Xiao et al., 1992) and carotenoids that possess gastric mucosa protection action (Kamath et al., 2008)

CONCLUSION

In summary, essential oil of *Momordica charantia* essential oil significantly inhibited ulcer lesion and ulcer length induced by HCl/ethanol. We believed that *M. charantia* possessed the mucoproctective and cytoprotective effect in strengthening the defensive mechanism gastric mucosal since it significantly reduced the gastric lesion area induced by HCl/ethanol. In term of histopathological changes, *Momordica charantia* showed significantly difference in edema, congestion, hemorrhage and necrosis features when compared with negative control and positive control group. Furthermore, *Momordica charantia* also exhibited its anti ulcer effect in dose dependent manner.

ACKNOWLEDGEMENT

First and foremost, I would hereby emphasize greatest gratitude and appreciation to my supervisor, Associate Professor Dr Muhd Nadzrul Hakim, for his never ending guidance, encouragement, advice, constructive comments and his willingness to help throughout the whole project. It was a great experience for me having this opportunity to do this study under his supervision. To Malarvili Selvaraja, for his kindness in giving advises on experiment being done and willing to share his experience with me.

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APPROVAL

It is hereby certified that I have read this project paper entitled Antiulcerogenic effect of *Momordica charantia* on HCl/ethanol induced rats by Mohd Nasier bin Kamaldin, and in my opinion it is satisfactory in term of scope, quality and presentation as a fulfillment of the requirements for the course BMS 3999.

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(Supervisor)

Date: 28th April 2010

DECLARATION

I hereby declare that the thesis is based on my original work except for the quotations and citations, which have been duly acknowledged.

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MOHD NASIER BIN KAMALDIN

Matric No: 135776

Date: 28th April 2010

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
ºC	degree Celsius
±	Plus minus (varied)
ANOVA	Analysis of Variance
GI	Gastrointestinal
HCl	Hydrochloric Acid
EtOH	Ethanol
PPI	Proton pump inhibitor
g	gram
H. pylori	*Helicobacter pylori*
NSAIDs	Nonsteroidal anti-inflammatory drugs
S.E.M	Standard Error Mean
M. charantia	*Momordica charantia*
w/v	Weight over volume
w/w	Weight over weight
UPM	Universiti Putra Malaysia

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Overview

Gastric ulcer is an ulcer that breach and craterlike like lesion in the mucosa of the stomach that extend through the submucosa into muscularis mucosa or deeper (Kumar *et.al,* 2003). It is also a local defect at excavation of the mucosal surface of the stomach and is occupied by the formation of inflammatory necrotic tissue. Gastric ulcer being more prevalent gastrointestinal disorder nowadays, it is caused by many factor. Ulcer disease results from an imbalance between aggressive factors and the ability of the gastroduodenal mucosal to protect and heal itself. Acid and pepsin secreted by the stomach are key 'aggressive elements' and are generally required for ulcers to develop (Hong, 2002). *Helicobacter pylori* and NSAIDs are the principal causes of impaired mucosal resistance and are the main causes of gastric ulcers (Hong, 2002).

Research shows almost 80 percent of gastric ulcers cases are caused by infection of bacterium *Helicobacter pylori* (Jefferson, 2005). Ulcerogenic drugs such as NSAIDs like aspirin and ibuprofen, factor imbalance defenses and offenses and also modern lifestyle such as alcohol and caffeine consumption, smoking also contributed for increasing incidence of gastric ulcer. NSAIDs are commonly used to treat pain and inflammation (Ivey, 1988). But NSAIDs also produce a broad range of toxic effect, frequently causing gastrointestinal (GI) toxicity that result in ulceration, bleeding and perforation of stomach (James, 2003). The prevalence of ulcer makes the clinical practitioner and researcher to concern for produce and investigate more drugs for treating ulcer, both herbal and synthetic.

The conventional drugs used in the treatment of gastric ulcer include histamine (H2) receptor antagonists, proton pump inhibitors, antacids and anticholinergics. However, most of these drugs contribute various undesirable side effects and drug interactions (A. Prakash, 1998). Herbal medicine has long been practiced outside of conventional medicine, furthermore herbal medicine is becoming more mainstreams as up to date analysis and research showed their value in the treatment and prevention of disease. In developing countries all over the world, 80% of population continues to use traditional medicine in primary medical problems. Nowadays, use of herbal medicine in gastric ulcers is well documented and it is believed to have fewer or no side effects compared to conventional drugs.

Many different species with ethnopharmacological background for treating gastric ulcer have been screened in recent years and some of these studies have produced encouraging results on plant like *Peperomia pellucida* (Erazuliana, 2007), *Allophylus serratus* (Dharmani, 2005), *Cardiospernum halicacabum* (Sheeba, 2005), *Kaempferia* *parviflora* (Rujjanawate, 2005), *Ocimum suave* (Tan, 2002), *Commiphora molmol* (Al-Harbi, 1997) and many more.

One such plant is *Momordica Charantia*, used in traditional medicine against ulcer. The *Momordica Charantia* or also known as bitter gourd has excellent medicinal virtues (Gurbuz, 1999: Yesilada, 1998). The fruits of *Momordica charantia* are reported to possess wide range of pharmacological activities such as hypoglycaemic (Zheng *et al*., 2005), antidiabetic (Sathishsekar and Subramanian, 2005a), antifungal (Schmourlo *et al*., 2005), inhibition of p-glycoproteins (Limtrakul *et al*., 2004), antihyperlipidemic (Chen and Li, 2005) and antioxidant effects (Sathishsekar and Subramanian, 2005). In this study, we are going to investigate anti ulcerogenic effect of *Momordica charantia* on HCl/ethanol induced rats. The reason for selecting *Momordica charantia* in this present study is because of its strong active compound and ethnopharmacological properties. This study was done in order to produce better and safer alternative for the treatment of gastric ulcer with implementation in food medicines especially as a supplement product. This study also believes to lead us in using the active compound of *Momordica charantia* to treat many diseases and would give beneficial effect to our health.

1.2 Hypothesis of the study

i. *Momordica charantia* possess a significant anti-ulcer activity in HCl/ethanol induced rats

ii. Histopathology of stomach that is induced by HCl/ethanol is significantly well protected by *M. charantia* seed oil

iii. Ulcer index and lesion length is well inhibited *M. charantia* seed oil

iv. The *M. charantia* seed oil exhibit its effect in a dose-dependent manner

1.3 Objectives

i. The investigate gastroprotective effect of *M. charantia* seed oil on HCL/ethanol ulcer induce rats

ii. To investigate *M. charantia* seed oil in inhibiting ulcer index and lesion length in pretreated rats

iii. To investigate the effect of *M. charantia* seed oil on histopathological study

CHAPTER 2

LITERATURE REVIEW

2.1 Anatomy of stomach

The stomach is a J-shaped enlargement of the gastrointestinal tract directly inferior to the diaphragm of the epigastric, umbilical, and left hypochondriac region of the abdomen. The stomach connect the esophagus to the duodenum, which the first part of the small intestine. The stomach has four main regions: the cardia, fundus, body and pylorus. The cardia surrounds the superior opening of the stomach. The rounded portion superior to the left of the cardia is the fundus. Inferior of the fundus is the large central portion of the stomach called the body. The region of the stomach connects to the duodenum is the pylorus. Pylorus has two parts: the pyloric antrum and the pyloric canal. When the stomach is empty, the mucosa lies in large folds, called rugae. The pylorus communicates with the duodenum via pyloric sphincter. The concave medial border of the stomach is caller lesser curvature, while the convex lateral border is called greater curvature (Tortora and Derrickson, 2006).

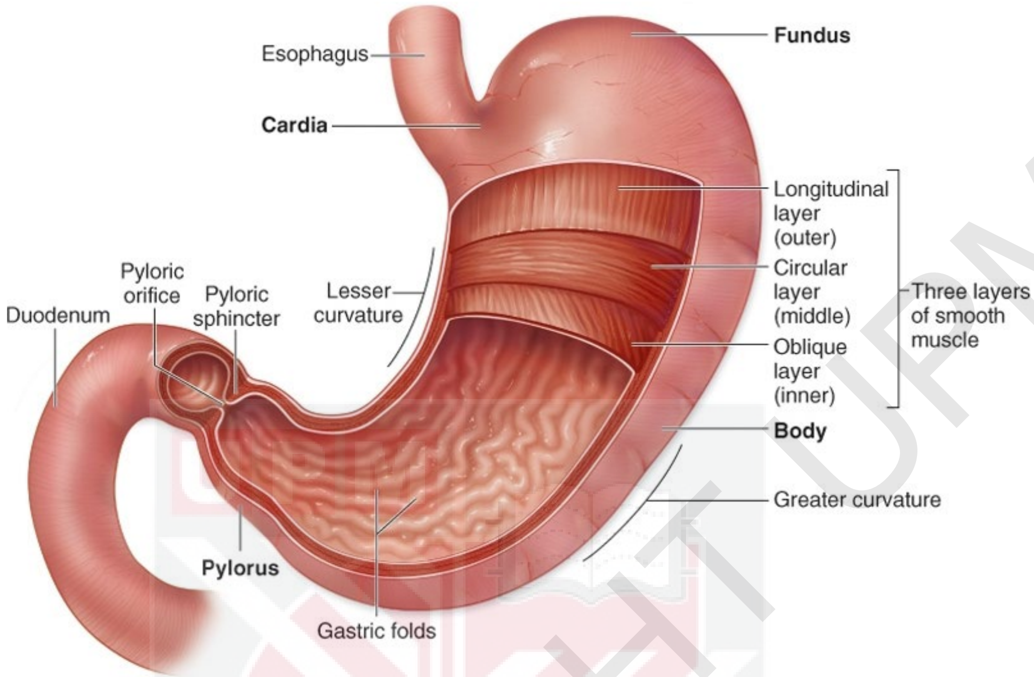


Figure 2.1: External and internal anatomy of stomach (Tortora and Grabowski, 1996)

2.2 Pathophysiology of the stomach

Stomach wall is composed of the same four layers as the rest of the GI tract which are: mucosa layer, submucosa, muscularis and serosa. The surface of the mucosa is a layer of the simple columnar epithelial cells called surface mucous cells. The mucosa contains a lamina propria (areolar connective tissue) and muscularis mucosae (smooth muscle). Epithelial cells extend down into the lamina propria, where they form columns of the secretory cells called gastric glands. The secretions from several gastric glands flow into each gastric pit and then into the lumen of the stomach. (Tortora and Derrickson, 2006)

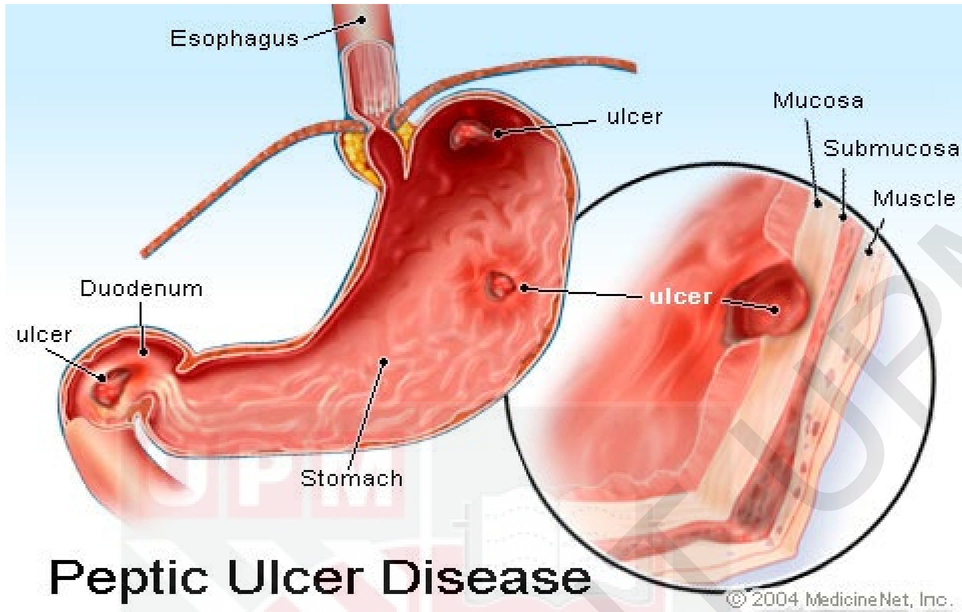
The gastric glands contain 3 typed of exocrine gland cells that secrete their products into the stomach lumen: mucous neck cells, chief cell and parietal cells. Bothe surface mucous cells and mucous neck cells secrete mucus. Parietal cells produce intrinsic factor (which needed for absorption of vitamin B_{12}) and hydrochloric acid, HCl. The chief cells secrete pepsinogen and gastric lipase. The secretions of the mucous, parietal and chief cells form gastric juice, which can be produced in totals of 2000-3000 mL per days. Gastric glands also included a type of enteroendocrine cells, the G cells, which secrete hormone gastrin into the bloodstream. The three additional layers lie deep to the mucosa: submucosa, muscularis and serosa. The submucosa of the stomach is composed of the areolar connective tissue. The muscularis composed 3 layer of smooth muscle: an outer longitudinal layer, a middle circular layer, and an inner oligue layer. The inner layer of the stomach, serosa composed of simple squamous epithelium (mesothelium) and areolar connective tissue. (Tortora and Derrickson, 2006)

2.3 Overview of the Peptic ulcer

2.3.1 Peptic ulcer

Peptic ulcers are chronic, most often solitary, lesion that occurs in any portion of gastrointestinal tract exposed to the aggressive action of acidic peptic juices (Robbins, Basic pathology). Peptic ulcers are most common in stomach (gastric ulcer) and duodenum (duodenal ulcer), the portion of the small intestine closest to the stomach. The mechanisms of injury differ distinctly between duodenal and gastric ulcers based on the pathophysiology of peptic ulcer disease. Peptic ulcers are defined histologically as a breach in the mucosa that extends through muscular mucosa into deeper submucosa or deeper (Robbins, Basic pathology). Peptic ulcer is illness that affects a considerable number of people in the world and it is caused by several factors like *Helicobacter pylori* (*H pylori*) infection, ingestion of non-steroidal anti-inflammatory drugs and physiological stress.

The pathophysiology of peptic ulcer has been reported to be due to an imbalance between offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion and cell shedding (Goel and Bhattacharya, 1991). Main causes of peptic ulcers are etiologically related to infection with *Helicobacter pylori* or use of nonsteroidal anti-inflammatory drugs (NSAIDs). Mechanisms resulting in improved anti-*H. Pylori* therapy includes better delivery of antimicrobials to the sites of infection, enhancing local immunity or by reducing the mucus barrier to topical therapy. Mechanisms to prevent NSAID ulcers include less damaging NSAIDs, reduction in acid secretion or replacement of mucosal prostaglandins (Hong Lu, 2002).



Figured 2.2: Peptic ulcer (Adapted from MedicineNet.inc, 2004)

2.3.2 Epidemiology of peptic ulcer

Peptic ulcers are relapsing lesion that are most often diagnosed in middle aged to older adults but they may first become evident in young adults life. People with peptic ulcer often appear without obvious precipitating influences and then may then heal after weeks to months of active disease. However, the possibility to develop peptic ulcer remain because of recurrent infection of *H. pylori*. Thus, it is difficult to obtain accurate data on the prevalence of active disease. For both man and women, the lifetime risk of developing peptic ulcer disease is about 10 %. Genetic seem to have little or no role in the causation of peptic ulcer. Duodenal ulcers are more common in person with alcoholic cirrhosis, chronic obstructive pulmonary disease, chronic renal failure, and hyperthyroidism (Robbins, 2006).

2.3.3 Pathogenesis of peptic ulcers

Pathogenesis of peptic ulcer remains murky. The complex and multifactorial pathogenesis of peptic ulcer has been studied over several decades, and the scientist discover that peptic ulcer disease has centered on an imbalance between aggressive gastric luminal factors acid and pepsin and protective mucosal barrier function (Alkohfahi and Atta, 1999). There are two condition key for the development of peptic ulcer, first is *H. pylori* infection, which has strong causal relationship with peptic ulcer development and second is mucosal exposure to gastric acid and pepsin. It is best perhaps to consider that peptic ulcers are created by an imbalance between the gastroduodenal mucosal defenses and the aggressive force that overcome such defenses (Robbins, 2006).

H. pylori infection is the most important condition in the pathogenesis of peptic ulcer. *H. pylori* infection causes the majority of duodenal (90–95%) and gastric ulcers (60–70%). The lifetime risk for developing a peptic ulcer among those with *H. pylori* infection is in the range of 1 in 6. *H. pylori* infection is typically acquired in childhood and clinical disease only occurs after a long latent period during which progressive gastroduodenal damage occurs (Hong Lu, 2002). *H. pylori* infection elicits an inflammatory response characterized by infiltration of both acute and chronic inflammatory cells, which tend to impair mucosal integrity. *H. pylori*, the host and the environment interact to produce clinical disease. Ulcers tend to form at the sites of maximum inflammation. The permanent cure of peptic ulcers by eradication of the infection was the ultimate proof which show that *H pylori* as the main cause of ulcer disease (Peitz and Malfertheiner *et al*., 2000). Eradication of *H. pylori* usually with anti-secretory agents such as proton pump inhibitor and antibiotics is a primary aim of therapy in peptic ulcer disease and usually results in complete healing of the ulcer (Benckiser, 2008).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the major cause of peptic ulcer disease in person who does not have *H. pylori* infection. NSAIDs was thought to induce gastric damage by exerting mechanism such as topical injury by ion trapping (Davenport *et al*., 1969) and reduction of mucus gel hydrophobicity (Romero *et al*., 1995). NSAIDs induce GI mucosal injury by direct toxic effects and by reducing mucosal prostaglandins which play an important role in defense mechanisms and repair processes. NSAIDs were shown to damage the gastric mucosa predominantly by the suppression of prostaglandin synthesis (Wallace *et al*., 2008). NSAIDs inhibit cyclooxygenase (COX), rate-limiting enzyme required for the conversion of arachidonic acid to prostaglandins. Two COX isoforms have been identified and referred as COX-1 and COX-2. The inducible COX-2 is an important regulator to generate prostaglandins that mediate inflammation, whereas the COX-1 is responsible for maintenance of the integrity of gastric mucosa and platelet aggregation (Robbins, Basic pathology). Chronic administrations of NSAIDs cause gastroduodenal mucosal erosions in approximately 35–60% of patients, gastric or duodenal ulceration in 10–25% of patients and severe complications, such as gastrointestinal hemorrhage or perforation in 1% of patients (C.J. Hawkey, 1990).

Prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation. The mechanism is still very poorly understood (Rao *et al*., 2000). Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage is caused by physical, chemical and psychological factors that leads to gastric ulceration in human and experimental animals (Rao *et al*., 1999). The evidence which shows stress as a contributory factor was the rise in bleeding gastric ulcers in folk population after a severe earthquake in Japan (Aoyama *et al*., 1998). The involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration (Sairam *et al*., 2001)

Gastric hyperacidity may strongly ulcerogenic. The excess production of gastric acid from a tumor in person with Zollinger-Ellison syndrome causes multiple peptic ulceration in the stomach, duodenum and even in jejunum (Robbins, Basic pathology). Other causes of peptic ulcer include pathologic hypersecretory states (e.g. Zollinger-Ellison syndrome, mast cell disease) and other infections like herpes simplex (Hong Lu, 2002). Gastric acid hypersecretion and acid overload in the duodenum leads to development of metaplasia in the duodenal bulb which is essential for *H pylori* colonization of duodenal epithelium, because colonization is exclusive and specific to gastric epithelial cells. After *H pylori* colonization, the inflamed duodenal mucosa becomes more susceptible to peptic ulceration. *H pylori* also infect neural pathways, with functional disruptions of antral-fundic neural connection that is responsible for regulation of acid production. The impairment of inhibitory neural control, in association with hypergastrinemia, leads to further increase of acid output in patients with duodenal ulcer (Chitajalu *et al*., 1991)

2.3.4 Clinical features of peptic ulcers

Most peptic ulcer cause epigastric pain often describe as burning, but significant minority first come to light with complication such as hemorrhage or perforation. The pain tends to be worse at night and occurs usually 1-3 hours after meals during the day. Nausea, bloating, vomiting, belching and weight loss are additional manifestation. Bleeding occurs in as many as one-third of the patient may be life threatening. Bleeding may be accompanied by hematemesis, melena, dizziness and diarrhea. Perforation is less frequent than bleeding, with an incidence of around seven to ten per 100 000 (Gisbert, 2003; Gustavsson, 1992). Perforation occurs in about 5% of the patients but accounts for two-third of death from this disease. Malignant transformation occurs in 2% of patient generally from ulcer in pyloric channel and is very rare with gastric ulcer. Peptic ulcers are recurrent lesion that often impairs the quality of life than shorten it. Nevertheless, with present ulcer therapy, most ulcer victim can be help if not cured and they are usually not need for surgery. (Robbins, 2006)

2.4 Anti-ulcer drug

Antiulcer drugs are a class of drugs used to treat ulcers in the stomach and the upper part of the small intestine. Gastric and duodenal ulcers are caused by Helicobacter pylori infections treated with combination treatments. Anti *H. pylori* therapy consists of the combination of antibiotics often with antisecretory drugs. Triple therapy is a legacy therapy consisting of a proton pump inhibitor (PPI) and two antibiotics combination of amoxicillin, clarithromycin or metronidazole (Hong Lu, 2002). Drugs used in treatment of peptic ulcer can be divided into two: agent that reduce intragastric acidity and agent that promote mucosal defense. (Katzung, 2007)

2.4.1 Agent that reduce intragastric acidity

2.4.1.1 Antacid

Antacids have been used centuries in treatment of patient with acid-peptic disorder. Antacids are weak bases that neutralize gastric acid by react with gastric hydrochloric acid to form a salt and water. Although their principal of action is reduction of intragastric acidity, they may also promote mucosal defense mechanism through mucosal stimulation of mucosal prostaglandin. The most commonly used antacids include salts of aluminum hydroxide, magnesium hydroxide, calcium or sodium bicarbonate. The acid neutralization capacity among different antacid is highly variable, depend on rate of dissolution, water solubility, rate of reaction with acid and the rate of gastric emptying (Katzung, 2007).

2.4.1.2 H_2 blockers

H_2-receptor antagonists (commonly known as H_2 blockers) were the most commonly used drugs in the world. H_2 blockers revolutionized treatment of peptic ulcer, healing ulcer and keeping them in remission when given as maintenance therapy (Collen and Benjamin, 1991). The H_2 blockers exhibit competitive inhibition at the parietal cell H_2 receptor and suppress stimulated acid secretion in a linear and dose dependant manner. They are very selective and do not affect other receptor. The volume of gastric secretion and the concentration of pepsin also reduced (Katzung, 2007). Four H_2 blockers are in clinical use are ranitidine, cimetidine, famotidine and nizatidine.

2.4.2 Mucosal protective agent

2.4.2.1 Sucralfate

Sulfacrate is a salt of sucrose complexed to sulfated aluminum hydroxide. In water or acidic solution it forms a viscous paste that binds selectively to ulcer or erosion for up to 6 hours. Sucralfate has limited solubility, breaking down into sucrose sulfate and an aluminum salt. Less than 3 % of intact drug and aluminum absorbed from the intestinal tract and the remainder is excreted in the feces. It is believed that negatively charged sucrose binds to positively charged proteins in the base of ulcer, forming a physical barrier that restrict damage and stimulates mucosal prostaglandin and bicarbonate secretion (Katzung, 2007).

2.4.2.2 Prostaglandin analogs

The human gastrointestinal mucosa synthesizes a number of prostaglandins. Misoprostol, a methyl analog of PGE_1, has been approved for gastrointestinal condition. Misoprostol has both acid inhibitory and mucosal protection properties. Misoprostol stimulates mucus and bicarbonate secretion and enhance mucosal blood flow. It binds to a prostaglandin receptor on parietal cell, reducing histamine stimulated cAMP production and causing modest acid inhibition (Katzung, 2007). Misoprostol is approved only for the prevention of NSAID-induced gastric ulcer. The adverse effects of misoprostol are diarrhea and cramping abdominal pain. This is because misoprostol stimulate uterine contraction and it should not be use during pregnancy.

2.5 Bittergourd *(Momordica charantia)*

2.5.1 Overview of *Momordica charantia*

The bittergourd (*Momordica charantia*) belong to the family Cucurbitaceae is found throughout the tropics, probably has been used by human and their immediate ancestors species for at least a million years. *Momordica charantia* (bitter gourd) is a plant native to the semi-tropical climate of China, India, Asia and Africa. This plant traditionally used medicinal herbs as, anti-HIV, anti-ulcer, anti-inflammatory, antileukemic, anti-microbial, anti-diabetic, and anti-tumor (Taylor, 2002; Grover and Yadav, 2004) and is one of the most promising alternative medicines for the disease. Momordica means, "to bite" referring to the jagged edges of the leaf, which appear as if bitten. All parts of the plant, including the fruit, taste bitter. The fruit is oblong and resembles a small cucumber. The young fruit is emerald green that turns to orange-yellow when ripe. At maturity the fruit splits into three irregular valves that curl backwards and release numerous brown or white seeds encased in scarlet arils (Grover and Yadav, 2004). *Momordica charantia* has been used traditionally as medicine in developing countries like Brazil, China, Colombia, Cuba, Ghana, Haiti, India Mexico, Malaya, New Zealand, Nicaragua, Panama and Peru. Some of its common uses in most countries are for diabetes, as a carminative and in treatment of colics (Yesilada et al., 1999; Satyawati et al., 1987).

Figure 2.3: The *Momordica charantia* plant (Nasier, 2010)

2.5.2 Ethnobotanical uses of *Momordica charantia*

Country	Uses
Brazil	Abortifacient, anthelmintic, aphrodisiac, burn, catarrh, colic, dermatosis, diabetes, diarrhea, eczema, emetic, emmenagogue, emollient, fever, febrifuge, hemorrhoids, hepatitis, hypoglycemic, inflammation (liver), leprosy, leucorrhoea, leukemia, malaria, menstrual colic, pain, pruritus, purgative, rheumatism, scabies, skin, tumor, vaginitis, vermifuge, wound,
China	Aphrodisiac, cancer (breast), diabetes, food, glucosuria, halitosis, hematuria, polyuria, refrigerant
Colombia	Bite (snake), malaria
Cuba	Anemia, colitis, emmenagogue, fever, hepatosis, hypoglycemic, kidney (stone), sterility (female), vermifuge
Ghana	Aphrodisiac, dysentery, fever, gonorrhea
Haiti	Anemia, appetite stimulant, dermatosis, eye, fever, insecticide, laxative, liver, sk in, rage, rhinitis
India	Abortifacient, anthelmintic, bite(snake), contraceptive, diabetes mellitus, dysmenorrhea, eczema, emmenagogue, fat loss, fever (malarial), galactagogue, gout, hydrophobia, hyperglycemia, jaundice, kidney (stone), laxative, leprosy, leucorrhea, liver, piles, pneumonia, psoriasis, purgative, rheumatism, scabies, skin, tonic,

	vegetable
Mexico	Aphrodisiac, burn, diabetes, dysentery, purgative, scabies, sore, vermifuge
Malaya	Abdomen, asthma, burn, dermatosis, diarrhea, headache, scald, sprue, stomachache, vermifuge,
Panama	Cold, emmenagogue, diabetes, fever, gallbladder, hypertension, insecticide, malaria, pruritus
Peru	Colic, contusions, diabetes, diarrhea, emetic, emmenagogue, febrifuge, hepatitis, inflammation, lung, malaria, measles, purgative, skin (sores), suppurative, vermifuge, wound
Trinidad	Diabetes, dysentery, fever, hypertension, malaria, rheumatism, vermifuge

Table 2.1: Worldwide Ethnobotanical uses of *Momordica charantia* (Adapted from Leslie Taylor, 2002)

2.5.3 Phytochemistry of *Momordica charantia*

Momordica charantia contains biologically active compound that include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids (Raman and Lau, 1996). Several phytochemicals such as momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins and multiflorenol have been isolated (Husain *et al*., 1994; Xie *et al*., 1998;Yuan *et al*., 1999; Parkash *et al*., 2002).The hypoglycemic/ antihyperglycemic chemicals of *Momordica charantia* are a mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids (Raman and Lau, 1996) and these chemicals are concentrated in fruits of *momordica charantia* (Ali *et al*., 1993). There are two types of hypoglycemic substances in *Momordica charantia* with different time dependent effects. The first one with fast antihyperglycemic activity of around 1 h present in the aqueous and the residue after alkaline chloroform extraction of aqueous extract and another with a slow hypoglycemic activity in acidic wash of the chloroform extract remaining after alkaline water wash (Day et al., 1990). *Momordica charantia* also contain HIV inhibitory proteins like MRK29 (MW: 28.6 kDa), MAP30 (MW: 30,000 kDa) and lectin (Putnam and Tainer, 2000). Protein (MAP30) has potential for the treatment of HIV and a host of other infections. It would be better if MAP30 are used in combination with current antiretroviral drugs. The presence of trypsin inhibitors (Miura and Funatsu, 1995), elastase inhibitors (Hamato *et al*., 1995), guanylate cyclase inhibitors (Vesely *et al*., 1977; Takemoto *et al*., 1980) and alpha-glucosidase inhibitor like D-(+)-trehalose in momordica charantia are well reported (Matsuur *et al*., 2002).

CHAPTER 3

MATERIAL AND METHODS

3.1 Materials

Essential oil was obtained from the dried seeds by hydrodistillation using the Clevenger-type apparatus. Air-dried seeds of *Momordica charantia* were ground and subjected to hydrodistillation for 3 h by use of a Clevenger type apparatus. The essential oil was isolated and dried over anhydrous sodium sulfate.

3.2 Animals

Weaned male *Sprague –Dawley* rats aged 5-6 weeks and weighing 150-200g used in the study were obtained from the Animal House of the Faculty of Medicine and Health Sciences, University Putra Malaysia with ethics approval from the Animal Ethics Committee, UPM(ACUC) with reference no. UPM/FPSK/PADS/BR-UUH/00335. The animals were maintained in standard laboratory conditions (temperature 25 ± 2 ºC). The animals were fed on standard pellet diet. Food was withdrawn 24 h before induction of ulcer to the animals, but the animals were allowed free access to water.

3.3 Chemical and drug

- Distilled water
- Hydrochloride
- Ethanol
- Ranitidine
- Formalin
- Diethyl ether

3.4 Experimental design

3.4.1 Anti-ulcerogenic activity

The experiment was performed n 36 male Sprague Dawley rats (150-200g). The rats were divided randomly into six groups, each consist of 6 rats (n=6)

1. Normal group, not treated with anything
2. Negative control group, treated with distilled water and induced by ulcerogen
3. Positive control group, treated with ranitidine and induced by ulcerogen
4. Group -100% , treated with 100% momordica charantia essential oil and induced by ulcerogen
5. Group - 50%, treated with 50% momordica charantia essential oil and induced by ulcerogen
6. Group - 10%, treated with 10% momordica charantia essential oil and induced by ulcerogen

Antiulcer assay performed by using HCl/ethanol combination method. Acute gastric ulcers were induced by oral administration of 1 ml HCl/ethanol. Animal pretreated for 7 days with distilled water(negative control) and ranitidine (positive control – 100 mg/kg) and with momordica charantia essential oil in various doses (100%, 50% and 10%) according to their to their body weight (10 ml/kg) and respective to the groups. On day 7, after one hour of pretreatment, all groups of animals except for normal group fed orally with HCl/ethanol. Rats were deprived of food but allowed free access of water 12 hour before HCl/ethanol administration. One hour after induce ulcer, animals were sacrificed and the stomach was removed, opened along the greater curvature and sum of length of lesions (mm) was calculated and expressed as lesion index. The number of ulcers and the length of each ulcer were measured (Rao *et al*., 1990). The lesion saiz in mm was determined by measuring each lesion along its greatest diameter/length using transparent grid. The severity score according to Minano *et al*. (1987).

Scoring	Description
0	No pathological changes
1	Mucosal oedema and perechial haemorrhages
2	1-5 small ulcers (1-2 mm)
3	> 5 small ulcers or 1 medium ulcer (3-4 mm)
4	2 medium ulcers or 1 large ulcer (more than 4 mm)
5	Perforated ulcers

Table 3.1: Ulcer lesion score according to Minamo *et al*. (1987)

The ulcer indexes (UI) for each stomach were calculated using the following formula: UI = [Ulcerated area / Total stomach area] × 100, and the ulcer inhibition percentage for each group were calculated as:

Ulcer Inhibition (%) = [(U control-UItreated/UIcontrol)] X 100.

3.4.2 HCl/EtOH-induced gastric ulcers

The momordica charantia essential oil was administered orally to 24h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml EtOH + 1.7 ml HCl + 38.3 mlH_2O) p.o. (Mizui and Doteuchi, 1988). The animals were sacrificed and examined for gastric ulcers 60 min later

3.4.3 Histopathological studies

For histological examination, the stomach tissues were excised and rinsed with saline solution (0.9% sodium chloride) to remove blood and debris adhering to tissues. The tissues were then fixed in 10% formalin and processed the tissue in processor machine a through grade ethanol, xylene, and impregnated paraffin (Al-Howiriny, 2005). The fixative was removed by washing through running tap water overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into 5µm thickness and stained with hematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope for details.

3.5 Statistical analysis

Data was expressed as mean ± S.E.M. the results of ulcer scoring and scoring data of histopathology study was analyzed with non parametric statistical analysis by Kruskal-Wallis test, followed by Man-Whitney U test for comparison within the group. For the ulcer index data was analyzed with one way ANOVA, followed by Dunnets multiple comparison test post-hoc comparison of group means. For all tests, effect of probability of $p<0.05$ were considered significant.

CHAPTER 4

RESULT

4.1 Ulcer length

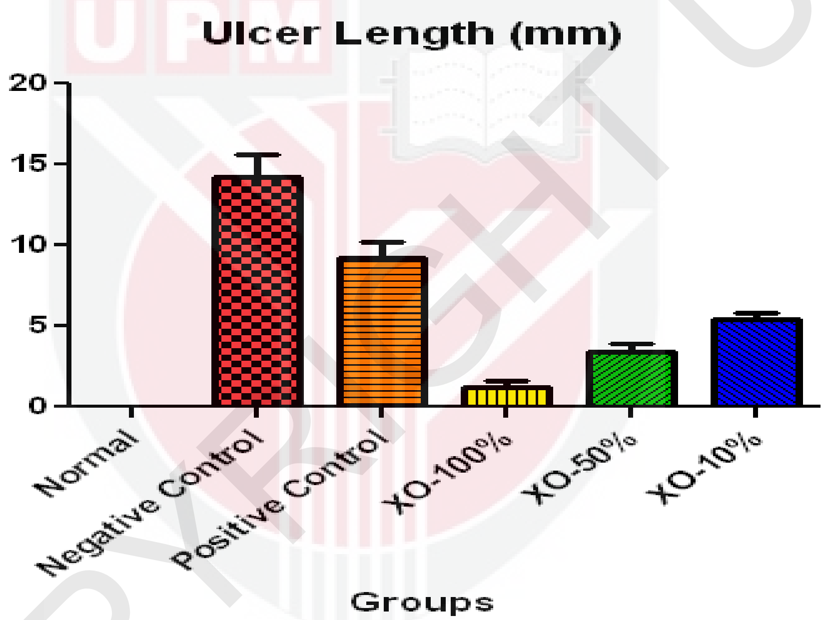


Figure 4.1: Effect of *Momordica charantia* L. seed essential oil on ulcer length induced by HCl/EtOH induced ulcer

Table 4.1: Effect of *Momordica charantia* L. seed essential oil on ulcer length induced by HCl/EtOH induced ulcer

Group	Ulcer length (mean±SEM)	Ulcer length inhibition (%)
Negative control	14.17±1.400	-
Positive control (Ranitidine)	9.167±1.078**	34.63
XO-100%	1.183±0.4254***	90.95
XO-50%	3.450±0.4877***	74.95
XO-10%	5.383±0.3842***	61.30

All values are mean±SEM, $n = 6$.
* $p < 0.05$ when compared to negative control
** $p < 0.01$ when compared to negative control
*** $p < 0.005$ when compared to negative control

As shown in Table 4.1, the *Momordica charantia* seed essential oil showed a dose dependent and significant anti-ulcerogenic activity in reducing ulcer length in stomach of HCl/ethanol animal induced model. Pretreatment with XO-100%, showed the most effective result in reducing ulcer length followed by XO-50%, XO-10% and positive control (ranitidine) respectively. XO-100% group significantly reduced ulcer length from 14.17±1.400mm^2 to 1.183±0.4254mm^2 ($p < 0.005$). XO-50% reduced ulcer length from 14.17±1.400mm^2 to 3.450±0.4877mm2 ($p < 0.005$). XO-10% reduced ulcer length from 14.17±1.400mm^2 to 5.383±0.3842mm2 ($p < 0.005$). Ranitidine reduced ulcer length from 14.17±1.400mm^2 to 9.167±1.078mm2 ($p < 0.005$).

4.2 Ulcer lesion

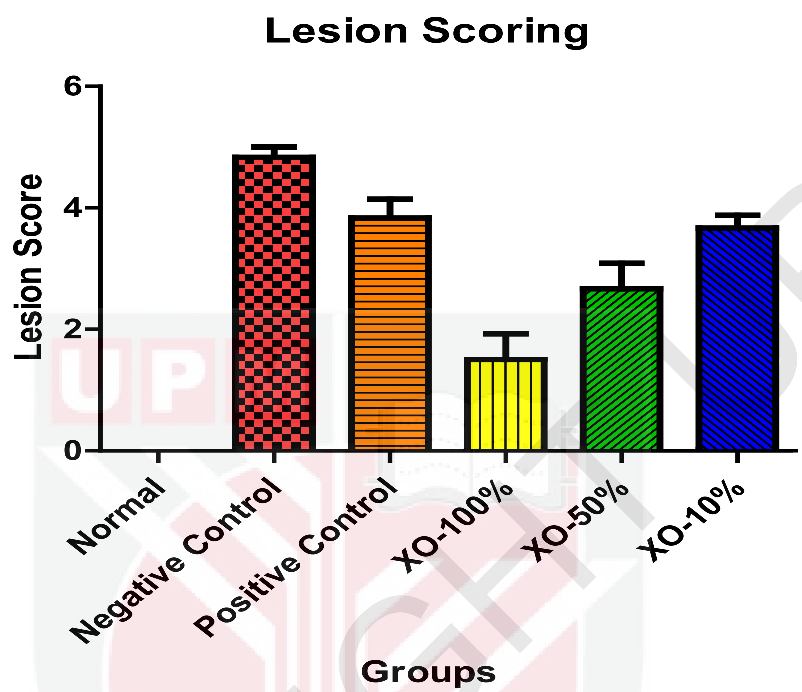


Figure 4.2: Effect of *Momordica charantia* L. seed essential oil on ulcer lesion induced by HCl/EtOH induced ulcer

Table 4.2: Effect of *Momordica charantia* L. seed essential oil on ulcer lesion induced by HCl/EtOH induced ulcer

Group	Ulcer lesion (mean±SEM)	Ulcer lesion inhibition (%)
Negative control	4.833±0.1667	-
Positive control (Ranitidine)	3.833±0.3073	20.69
XO-100%	1.500±0.4282***	68.96
XO-50%	2.667±0.4216***	44.82
XO-10%	3.667±0.2108	24.12

All values are mean±SEM, $n = 6$.
* $p < 0.05$ when compared to negative control
** $p < 0.01$ when compared to negative control
*** $p < 0.005$ when compared to negative control

As shown in Table 4.2, the *Momordica charantia* seed essential oil showed a dose dependent and significant anti-ulcerogenic activity in reducing ulcer lesion in stomach of HCl/ethanol animal induced model. Pretreatment with XO-100%, showed the most effective result in reducing ulcer leesion followed by XO-50%, XO-10% and positive control (ranitidine) respectively. XO-100% group significantly reduced ulcer lesion from 4.833±0.166mm^2 to 1.500±0.4282mm^2 ($p < 0.005$). XO-50% reduced ulcer lesion from 4.833±0.166mm^2 to 2.667±0.4216mm^2 ($p < 0.005$). XO-10% reduced ulcer lesion from 4.833±0.166mm^2 to 3.667±0.2108mm^2. Ranitidine reduced ulcer lesion from 4.833±0.166mm^2 to 3.833±0.3073mm^2.

4.3 Percentage of inhibition

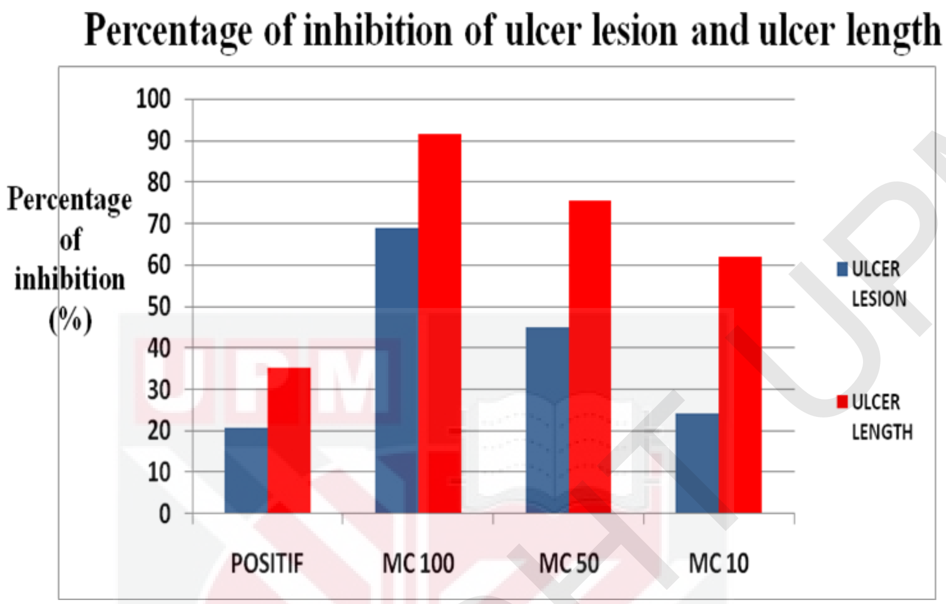


Figure 4.3: Effect of *Momordica charantia* L. seed essential oil on percentage of ulcer lesion and ulcer length induced by HCl/EtOH induced ulcer

As shown in Figure 4.3, the essential oil of *M. charantia* fruits showed a dose dependent and significant anti-ulcerogenic activity. At higher dose, 100% concentration of *M. charantia* seed essential oil, it showed maximum inhibition of ulcer length with the percentage of 90.95% and ulcer lesion with percentage of 68.96%. At 50%, it inhibited ulcer length by 74.95% and ulcer lesion by 44.82%. At 10%, it showed lowest inhibition of ulcer length by 61.30% and ulcer lesion by 24.12% if compared to previous group of treatment. Positive group inhibited ulcer lesion by 20.69% and ulcer length by 34.63%.

4.4 Histology of stomach

4.4.1 Normal group

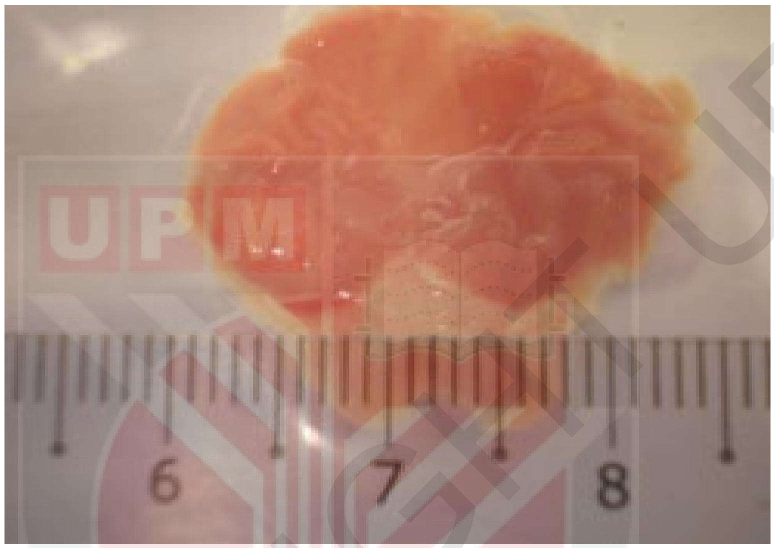
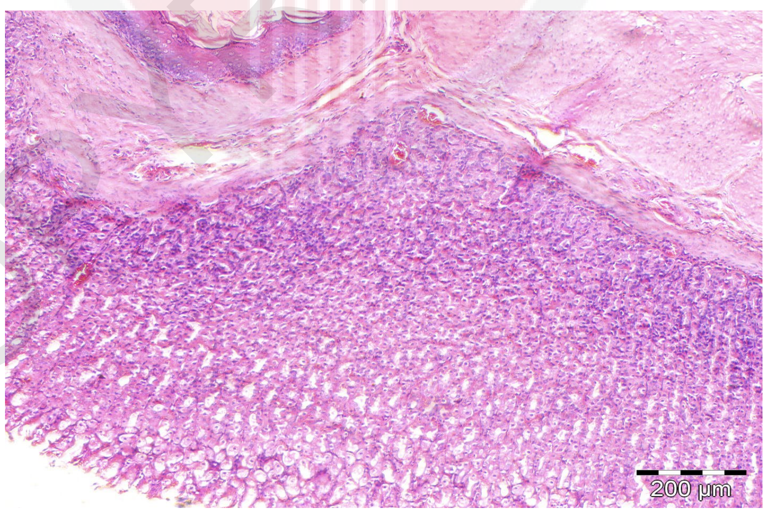


Figure 4.4: Normal stomach



NORMAL (10X)

4.4.2 Negative control group

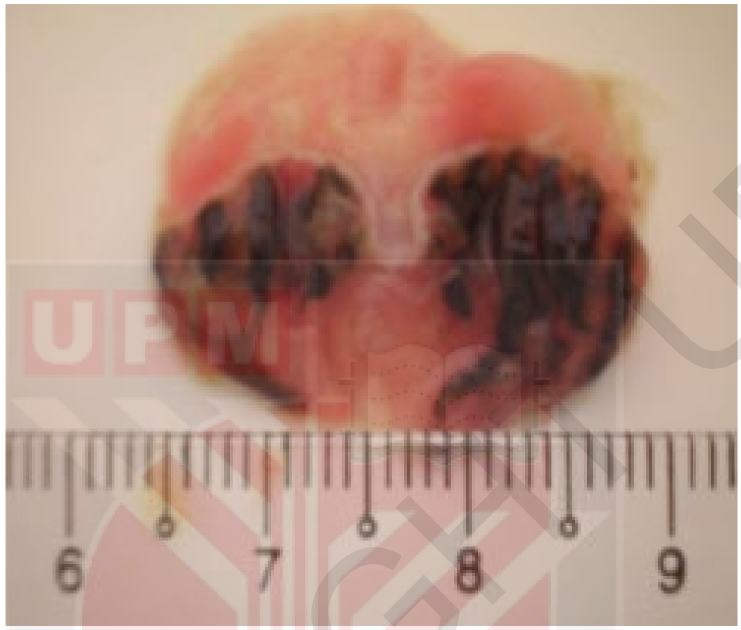
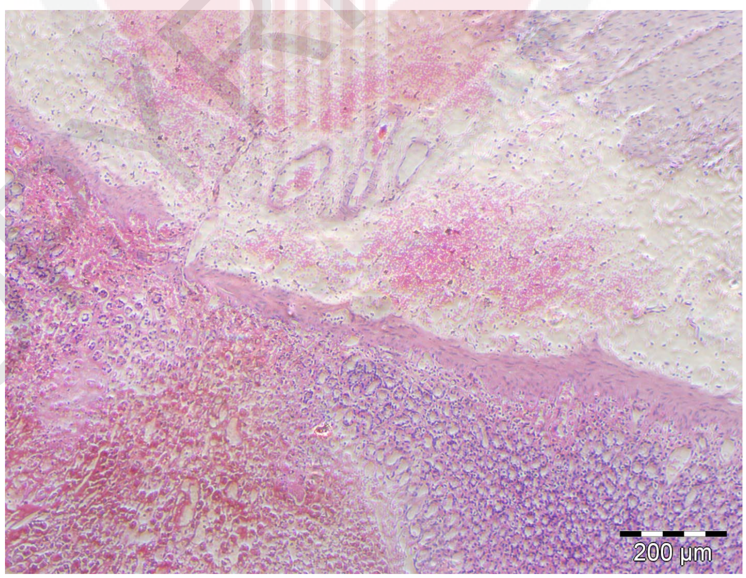


Figure 4.5: Stomach that induced by HCl/Ethanol



NEGATIVE CONTROL (10X)

4.4.3 Positive control group (ranitidine)

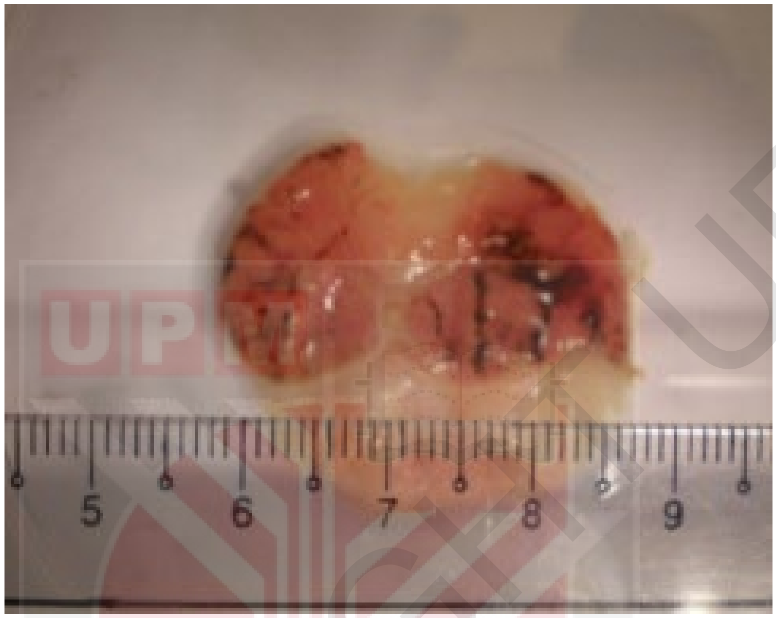


Figure 4.6: Stomach that treated with ranitidine

POSITIVE CONTROL (10X)

APPENDICES

Appendix A

Histological processes

Tissue fixation in 10% formalin

↓

Grossing - tissue sampling 3-5mm

↓

Processing – Tissue processor (16 hours)

↓

Embedding/Blocking

↓

Trimming

↓

Sectioning

↓

Fishing

↓

Staining

↓

Mounting

Appendix B

Tissue processing process

70 % alcohol
↓
80 % alcohol — Rinsing
↓
90 % alcohol
↓
100 % alcohol
↓
100 % alcohol — Dehydration
↓
100 % alcohol
↓
Chloroform I
↓
Chloroform II — Clearing
↓
Chloroform III
↓

Wax I → Wax II } Impregnation

Appendix C

Haematoxylin and eosin staining

Xylene I } 4 minutes

↓

Xylene II

↓

100 % alcohol

↓

95 % alcohol

↓

80 % alcohol } 2 minutes (Xylene II to 80 % alcohol)

↓

70 % alcohol

↓

Wash in running water } 2-5 minutes

↓

Hematoxylin (nucleus staining) } 20 minutes

↓

Wash in running water } 5 minutes

↓

Acid alcohol } 5 dips

↓

Wash in running water } 2 minutes

↓

Weak ammonia } 5 dips

↓

Wash in running water } 10 minutes

↓

Eosin (cytoplasm staining) } 4 minutes

↓

70 % alcohol

↓

80 % alcohol

↓

95 % alcohol

↓

100 % alcohol

↓

Xylene I

(70 % alcohol to Xylene I: } 2 minutes)

↓

Xylene II

↓

Mounting

Appendix D

Statistical analysis results for ulcer lesion and length

Table Analyzed	Ulcer length		
One-way analysis of variance			
P value	< 0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	6		
F	46.08		
R square	0.8848		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)			
P value			
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	849.8	5	170.0
Residual (within columns)	110.7	30	3.689
Total	960.5	35	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant P < 0.05?	Summary	95% CI of diff
Normal vs Negative Control	-14.17	18.07	Yes	***	-17.54 to -10.79
Normal vs Positive Control	-9.167	11.69	Yes	***	-12.54 to -5.794
Normal vs XO-100%	-1.183	1.509	No	ns	-4.556 to 2.190

Normal vs XO-50%	-3.450	4.400	Yes	*	-6.823 to -0.07692
Normal vs XO-10%	-5.383	6.866	Yes	***	-8.756 to -2.010
Negative Control vs Positive Control	5.000	6.377	Yes	**	1.627 to 8.373
Negative Control vs XO-100%	12.98	16.56	Yes	***	9.610 to 16.36
Negative Control vs XO-50%	10.72	13.67	Yes	***	7.344 to 14.09
Negative Control vs XO-10%	8.783	11.20	Yes	***	5.410 to 12.16
Positive Control vs XO-100%	7.983	10.18	Yes	***	4.610 to 11.36
Positive Control vs XO-50%	5.717	7.291	Yes	***	2.344 to 9.090
Positive Control vs XO-10%	3.783	4.825	Yes	*	0.4103 to 7.156
XO-100% vs XO-50%	-2.267	2.891	No	ns	-5.640 to 1.106
XO-100% vs XO-10%	-4.200	5.357	Yes	**	-7.573 to -0.8269
XO-50% vs XO-10%	-1.933	2.466	No	ns	-5.306 to 1.440

Table Analyzed	**Ulcer lesion**		
One-way analysis of variance			
P value	< 0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	6		
F	35.21		
R square	0.8544		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)			
P value			
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	92.92	5	18.58
Residual (within columns)	15.83	30	0.5278
Total	108.8	35	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant P < 0.05?	Summary	95% CI of diff
Normal vs Negative Control	-4.833	16.30	Yes	***	-6.109 to -3.557
Normal vs Positive Control	-3.833	12.92	Yes	***	-5.109 to -2.557
Normal vs XO-100%	-1.500	5.058	Yes	*	-2.776 to -0.2241
Normal vs XO-50%	-2.667	8.991	Yes	***	-3.943 to -1.391
Normal vs XO-10%	-3.667	12.36	Yes	***	-4.943 to -2.391
Negative Control vs Positive Control	1.000	3.372	No	ns	-0.2759 to 2.276
Negative Control vs XO-100%	3.333	11.24	Yes	***	2.057 to 4.609
Negative Control vs XO-50%	2.167	7.305	Yes	***	0.8908 to 3.443
Negative Control vs XO-10%	1.167	3.934	No	ns	-0.1092 to 2.443
Positive Control vs XO-100%	2.333	7.867	Yes	***	1.057 to 3.609
Positive Control vs XO-50%	1.167	3.934	No	ns	-0.1092 to 2.443
Positive Control vs XO-10%	0.1667	0.5620	No	ns	-1.109 to 1.443
XO-100% vs XO-50%	-1.167	3.934	No	ns	-2.443 to 0.1092
XO-100% vs XO-10%	-2.167	7.305	Yes	***	-3.443 to -0.8908
XO-50% vs XO-10%	-1.000	3.372	No	ns	-2.276 to 0.2759