STUDY OF THE EFFECT OF AQUEOUS EXTRACT OF (*HIBISCUS ESCULENTUS L.*) ON INDOMETHACIN-INDUCED GASTRIC MUCOSAL DAMAGE AND SECRETIONS

BY

MALAJIYA, IBRAHIM SALEH

DEPARTMENT OF HUMAN PHYSIOLOGY,
FACULTY OF MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA

MARCH, 2009

STUDY OF THE EFFECT OF AQUEOUS EXTRACT OF (*HIBISCUS ESCULENTUS* L.) ON INDOMETHACIN-INDUCED GASTRIC MUCOSAL DAMAGE AND SECRETIONS

BY

MALAJIYA, IBRAHIM SALEH MB,BS (ABU 2002)
(MSc./Med/57536/2005-06)

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA NIGERIA IN PARTIAL FULFILMENT FOR THE AWARD MASTER OF SCIENCE IN HUMAN PHYSIOLOGY

DEPARTMENT OF HUMAN PHYSIOLOGY,
FACULTY OF MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

MARCH, 2009

DECLARATION

I declare that the work in the thesis entitled "STUDY OF THE EFFECT OF AQUEOUS EXTRACT OF (*HIBISCUS ESCULENTUS L.*) ON INDOMETHACIN-INDUCED GASTRIC MUCOSAL DAMAGE AND SECRETIONS" has been performed by me in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria under the supervision of Professor M.A. Mabrouk and Dr. A.B. Adelaiye.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Malajiya, Ibrahim Saleh **Date**

CERTIFICATION

This thesis entitled "STUDY OF THE EFFECT OF AQUEOUS EXTRACT OF (*HIBISCUS ESCULENTUS L.*) ON INDOMETHACIN-INDUCED GASTRIC MUCOSAL DAMAGE AND SECRETIONS" by Malajiya, Ibrahim Saleh meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

______________________________ ____________________

Professor M.A. Mabrouk **Date**
Chairman, Supervisory Committee

______________________________ ____________________

Dr. A.B Adelaiye **Date**
Member, Supervisory Committee

______________________________ ____________________

Professor M.A. Mabrouk **Date**
Head of Department

______________________________ ____________________

Professor S. Nkom **Date**
Dean, Postgraduate School

DEDICATION

To my parents, my wife Maryam and our little daughter Fatima and all those who contributed to knowledge and the development of mankind.

ACKNOWLEDGEMENT

I am very happy to register my sincere appreciation and gratitude to all those who have contributed in many different ways towards the successful completion of this work. Without such contributions, the work would have not been a reality.

My great appreciation goes to my supervisors: Prof. M.A Mabrouk, Dr. A.B Adelaiye and the invaluable contribution of my teacher Prof. M.A.M.E Okasha.

To my mother, Hajiya A'ishatu, and father Alhaji Saleh Malajiya, for your consistent prayers, that have really shown me light in all I pursue, may ALLAH reward you abundantly. My wife, Maryam Sunusi and our little daughter Fatima have shown great deal of patience for the overstay in the laboratory during the course of the research work. My brothers Yakubu Saleh and Aminu Saleh for their moral support.

Dr. Rabiu Magaji Abdulsalam, Dr .Yusuf Tanko, Dr. Aliyu Mohammed and Dr. Alhassan Abdulwahab all of Department of Human Physiology, Ahmadu Bello University, Zaria deserve a special thank you for their contribution to the success of this work.

Other friends and or colleagues: Ahmad Lawan, Ibrahim Bako, Dr. Usman Bappah, Dr. Shehu Zailani, Ibrahim Shehu, Dr. M.D. Yusuf, Abdullahi Yaro, Dr. Sufyan Jibrin, Dr. Zaiyad Habib, Dr. Nisser Umar, Abdullahi Yaro, Muhammad Garba Magaji, Dr.

Suleman Auwal and others too numerous to mention, for your company and moral support.

I really appreciate the contributions of Mal. Adamu of Pharmacognosy Department for extracting the plant material, Dr. Aliyu Musa of Pharmaceutical Chemistry Department for the phytochemical screening, Mal. Ibrahim of Pharmacology and Clinical Pharmacy for the toxicity studies, Dr. Abdullahi Yaro, Department of Pharmacology, Faculty of Medicine, Bayero University, Kano for his valuable suggestions and supplying the animals. Others are Dr. Sa'ad Ahmad of Dept of Pathology, Ahmadu Bello University Teaching Hospital, Zaria who helped in the histopathological report. Mal. Ya'u of Human Physiology Department for taking care of the animals used in this study.

I sincerely thank you all.

MALAJIYA, IBRAHIM SALEH
March, 2009

ABSTRACT

Gastric ulcers are breakages in the continuity of the gastric mucosal epithelial lining, caused by imbalances between offensive and defensive factors of the gastric mucosa. The anti-ulcerogenic activity of the many plant products is reported due to an increase in the mucosal defensive factors rather than decrease in the offensive factors. *Hibiscus esculentus*, an evergreen shrub of the savannah region of Africa, but indigenous to the Nile, Mediterranean and India was investigated for its effects on gastric ulceration and secretion. A total of eighty (80) adult male albino rats were used in this study, which were divided into control, indomethacin (20mg/kg), extract (100 and 200mg/kg) and cimetidine treated groups. It was carried out using the Shay et al model of ulcers induction, with indomethacin as the necrotizing agent. Preliminary phytochemical analysis of the extract revealed the presence of flavonoids, glycosides and phenolics. The oral and subcutaneous LD_{50} study of the extract in mice was found to be above 5000mg.kg. Group 1 that serves as control were given only subcutaneous distilled water. There was a statistical significance $p<0.05$ in the ulcer index and preventive index respectively in group 2, and a statistical significance of $p<0.05$ in the extract and cimetidine treated groups when compared with the control group. Gastric secretion as affected by indomethacin is insignificant in this study ($p<0.05$). These include volume and titratable acidity. However, there is a marked decrease in acid output. Histopathological analysis of the tissues revealed normal histological architecture of the gastric mucosa in the control group. Deep ulcerations stretching to the mucosa with necrotized bases were observed in the indomethacin treated group whereas the extract treated group markedly reduced the incidence and severity of the ulcerations.

Keywords: *Hibiscus esculentus*, gastric mucosal integrity, phytochemicals, anti-ulcerogenic, offensive factors.

ABBREVIATIONS

LD_{50}	-	Lethal Dose
NSAIDs	-	Non steroidal anti-inflammatory drugs
BC	-	Before Christ
UNDP	-	United Nation Development Programme
ENS	-	Enteric Nervous System
CNX	-	Cranial Nerve 10
HCl	-	Hydrochloric Acid
H^+	-	Hydrogen ion
α	-	alpha
β	-	Beta
ATP	-	Adenosine Triphosphate
ECL	-	Enterochromaffin-Like
5HT	-	5 Hydroxytrpthamine
CO_2	-	Carbon IV oxide
H_2CO_3	-	Carbonic Acid
HCO_3^-	-	Bicarbonate ion
OH^-	-	Hydroxyl ion
H^+/K^+ ATPase	-	Hydrogen-potassium ATPase
NIH	-	National Institute of Health
DNA	-	Deoxyribonucleic Acid
IgA	-	Immunoglobulin A

IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
CDC	-	Centre for Disease Control
NO	-	Nitric Oxide
COx	-	Cyclooxygenase
PG	-	Prostaglandin
PGE_2	-	Prostaglandin E_2
Cy P450	-	Cytochrome P450
GERD	-	Gastroesophageal Reflux Disease
LFTs	-	Liver Function Tests
PPI	-	Protein Pump Inhibitors
SC	-	Subcutaneous
Meq/L	-	Microequivalent per liter

TABLE OF CONTENTS

Cover page		i
Title Page		ii
Declaration		iii
Certification		iv
Dedication		v
Acknowledgement		vi
Abstract		viii
Abbreviations		x
Table of contents		xii
List of tables		xvi
List of plates		xvii
List of appendices		xviii

Chapter One

Introduction		1
1.1	Statement of research Problems	1
1.2	Justification of the Study	1
1.3	Benefits of medicinal plants	2
1.4	OKRA (*Hibiscus esculentus*)	3
1.5	Aims and objectives	4

Chapter Two

Literature Review		6
2.1	Physiologic-Anatomy of the Gastrointestinal Tract	6
2.1.1	Blood Supply	8
2.1.2	Nerve Supply	8
2.2	Anatomy of the Rat Stomach	10
2.3	Functional Anatomy of the Gastroduodenal Mucosa	10
2.3.1	Parietal or Oxyntic Cells	12
2.3.2	Chief Cells	13
2.3.3	Mucous Neck Cells	14
2.3.4	Hormone Secreting (Endocrine) Cells	14
2.4	Gastric Secretion	14
2.4.1	Mechanism of Gastric Acid Secretion	15
2.4.2	Mechanism of Hydrochloric Acid Secretion	16
2.4.3	Pepsinogen Secretion	17
2.4.4	Feedback Inhibition of Gastric Acid Secretion	18
2.4.5	Inhibition of Gastric Secretion by Intestinal Factors	19
2.4.6	Regulation of Gastric Acid Secretion	20
2.5	Peptic Ulcer Disease (PUD)	21
2.5.1	Pathophysiology of Peptic Ulcer Disease	24
2.5.2	Diagnosis of Peptic Ulcer disease	35
2.5.3	Management of Peptic Ulcer Disease	38
2.5.4	Drug Management	39

2.5.5 Surgical Management 45

Chapter Three

Materials and Methods 48

3.1 Materials 48

3.1.1 Animals 48

3.1.2 Drugs and Chemicals 49

3.1.3 Plant Material 50

3.2 Methods 50

3.2.1 Extraction of the Plant Material 50

3.2.2 Identification of Chemical Constituents 51

3.2.3 Acute Toxicity Studies 53

3.2.4 Induction of Gastric Ulceration 53

3.2.5 Collection of Gastric Secretion 54

3.2.6 Analysis of Gastric Juice 56

3.2.7 Statistical Analysis 57

Chapter Four

Results 58

4.1 Phytochemical Screening 58

4.2 Acute Toxicity Studies 58

4.3 Gastric Mucosal Integrity Studies 58

4.3.1 Control Group 58

4.3.2 Indomethacin-treated Group 58

4.3.3 Cimetidine plus indomethacin-treated Group 60

4.3.4 *Hibiscus esculentus* extract plus indomethacin-treated Group 60

4.4 Gastric Secretion Studies 60

4.4.1 Indomethacin-treated Group 60

4.4.2 Cimetidine-treated Group 61

Chapter Five

Discussions 71

5.1 Effects of Indomethacin on Gastric Mucosa and Gastric Secretion Parameters 71

5.2. Effects of Cimetidine as Standard anti-ulcer Drug on Mucosal Damage and Gastric Secretion Parameters 74

5.3. Effects of *Hibiscus esculentus* Aqueous Extract on Mucosal Damage and Gastric Secretion Parameters 76

Chapter Six

Summary, Conclusions and Recommendations 81

References 82

Appendix 95

LIST OF TABLES

Table 1:	Ulcer index (mm) and preventive index (%) for control and I indomethacin treated rats	59
Table 2:	Ulcer index (mm) and preventive index (%) for control, indomethacin and cimetidine treated rats	61
Table 3:	Summary of the different parameters in gastric ulcers study	62
Table 4:	Summary of the different parameters studied for 3 hours gastric secretion for control and Indomethacin-treated groups	63
Table 5:	Summary of the different parameters studied for 3 hours gastric secretion for control, Indomethacin and cimetidine treated groups	64
Table 6:	Summary of the different parameters studied for 3 hours gastric secretion for control, Indomethacin, extract and cimetidine-treated groups	65

LIST OF PLATES

Plate 4.1:	Normal histological architecture of gastric mucosa	66
Plate 4.2:	Ulceration of superficial gastric epithelium and enlargement of gastric pits evident	67
Plate 4.3:	Severe ulceration and necrosis of superficial gastric epithelium and deep ulceration extending through the mucosa	68
Plate 4.4:	Severe necrosis and ulceration of superficial gastric epithelium; enlargement of gastric pits plus deep ulcerations stretching through the mucosa	69
Plate 4.5:	Severe ulceration and necrosis of superficial gastric epithelium plus enlargement of gastric pits and deep ulcerations stretching through the mucosa	70

LIST OF APPENDICES

Appendix 1:	Ulcer Index and preventive index for control and indomethacin-treated rats	95
Appendix II:	Ulcer Index and preventive index for control, indomethacin and cimetidine-treated rats	96
Appendix III:	Ulcer Index and preventive index for control, indomethacin and Okra extract-treated rats	97
Appendix IV:	Parameters of gastric secretion at 3 hours for control and indomethacin-treated rats	98
Appendix V:	Parameters of gastric secretion at 3 hours for control, indomethacin, Okra extract and cimetidine-treated rats	99

CHAPTER ONE

INTRODUCTION

1.1 STATEMENT OF RESEARCH PROBLEMS

Peptic ulcers are breakages in the continuity of the mucosal epithelial lining of the stomach, duodenum and at times lower oesophagus and Merkel's diverticulum. It can also occur in areas of ectopic gastric tissue, and in small and large bowels in Zollinger-Ellison syndrome (Falase and Akinkugbe, 2000).

Alterations in mucosal protective factors induced by *Helicobacter pylori* are significant cofactors in the formation of peptic ulcer disease. Cigarette smoking, alcohol use and NSAIDs are also factors that impair mucosal protective factors (Belaice *et al*, 2002). In Nigeria, studies showed prevalence rates of 78.5 % of patients that presented with dyspepsia in Maiduguri (Mustapha *et al*., 2007) and a prevalence of about 84% in Kano (Tijjani *et al*., 2005).

1.2 JUSTIFICATION OF STUDY

(i) The study of aqueous extract of *Hibiscus esculentus* may provide us with a bioactive compound for drug development.

(ii) Exclusive use of aqueous extract of *Hibiscus esculentus* may be helpful in the management of peptic ulcer diseases due to its availability and wide range of uses by many people of low socioeconomic status who are more susceptible to peptic ulcer.

(iii) Elucidating the possible mechanism of action of the extract.

Medicinal herbs constitute the cornerstone of traditional medicinal practice worldwide. These herbs are relatively cheap and available and their use depends on ancestral experience (Main-Bettolo, 1980).

The growing interest in herbal medicine currently witnessed globally in most circles is viewed as a welcome development. In many African countries today, a place of prominence is given to herbal medicine both for curative and nutritional purposes (Akah, 1994).

1.3 BENEFITS OF MEDICINAL PLANTS

Many plant uses in traditional medicine have been proven useful in drug development and modern medicine. Drugs developed from plants or plant parts can be classified into three (3) (Sofowora, 1993).

(i) Drugs from plant origin used for treatment of different ailments in modern medicine;

(ii) Drugs which are synthetic derivatives of natural products;

(iii) Drugs used in orthodox medicine today which are directly derived from traditional remedies for the same treatment as the traditional medicine e.g. digoxin from digitalis.

Hence, a close scientific study of many such plants used locally for both nutritional purposes and traditional medicine is of paramount importance. *Hibiscus esculentus* is being used predominantly for nutritional purposes and to some extent for different medical purposes. Northern Nigeria is where its usage has cut across virtually all social strata of the society. However, no work has been done on its activity on peptic ulcer disease.

Due to the fact that *Hibiscus esculentus* soups and gravies are highly consumed by many people, it is desirable to study its exclusive use for possible antiulcer property.

1.4 OKRA (*Hibiscus esculentus*)

Okra, that is, *Hibiscus esculantuna*, is also called *Abelmoscus esculantuna* is an evergreen shrub about 1 to 1.5m high, indigenous to the Nile, Mediterranean, Balkans and India. It is called 'kubewa' (Hausa), 'ekewere (Igbo) and "ilasado (Yoruba). It is sliced, boiled or fried and eaten as food. It has a high mucilage content and as such used in soups and gravies.

It is found in its wild state on the alluvial banks of the Nile, and the Egyptians were the first to cultivate it in the basin of the Nile (12^{th} century BC). It was propagated then through North Africa to the Mediterranean, the Balkans and India. Even today, ground okra is used in West Africa to make a local soup made from dried and ground okra baobab leaves or rosette (from a UNDP report). Okra is a wide source of many nutrients including fiber, Vit. B6, and folic acid.

Okra is a multibranched evergreen herb, erect, coarse, robust, growing up to 1 to 1.5m tall. It has pod fruits that are beaked, ridged, more or less oblong hairy capsule that dehisces longitudinally. The fruits are light green. Sometime purple in colour (Gill, 1992).

Some constituents of the okra tree include the following:
The Pod is the fleshy part of the fruit with the seeds inside. This edible portion of the tree contains approximately 86.1% water, 2.2% protein, 0.2% fat, 9.7% carbohydrate, 1.0% fibre and 0.8% ash.

It also plays a role in the prevention of constipation by absorbing water and ensuring bulky stools. This action is seen on the okra slippery mucilage which many people abhor. It also helps in preventing hypercholesterolaemia by binding excess cholesterol. It is reported to also have antipyretic and antigonorrhoel property (Sylvia, 2004).

1.5 AIMS AND OBJECTIVES

This study is intended:

(i) To investigate whether aqueous extract of Hibiscus esculentus possess antiulcer effects;

(ii) To examine the role of Hibiscus esculentus on basal gastric acid secretion;

(iii) To investigate/evaluate the effect of aqueous extract of Hibiscus esculentus on the possible protection against peptic ulcer predominantly by its use alone.

The following parameters were studied:

a) Ulcer index

b) Preventive index

c) Volume of gastric juices

d) Titritable acidity

e) Total acid output

f) Histopathological studies

CHAPTER TWO

LITERATURE REVIEW

2.1 PHYSIOLOGIC-ANATOMY OF THE GASTROINTESTINAL TRACT

This tract refers to the entire hollow tubular system extending from the mouth to the esophagus and through the stomach, small and large intestine to end at the anus. The stomach is a muscular bag forming the widest and most distensible part of the digestive tube. It is connected above to the lower end of the esophagus and below to the duodenum. It begins at the distal end of the esophagus, enters the abdomen by piercing the diaphragm opposite the 10th thoracic vertebra (T10), to the left of the midline. The stomach is relatively fixed at its esophageal end and its pyloric end, between these positions it's quite mobile. It may be J-shape or steer horn-shaped (Guyton and Hall, 2000).

The stomach lies obliquely in the upper and left part of the abdomen, most of it lies under cover of the costal margin. It is distensible and about 25cm long. The stomach has two orifices, the cardiac orifice which lies behind the left 7th costal cartilage at the level of T11 vertebra and the pyloric orifice that open into the duodenum. Its position when the stomach is empty and supine is at the level of the lower border of L1 vertebra. Two curvatures mark the surface of the stomach; the lesser curvature is concave and forms the right border of the stomach; a sharp angular notch at the lesser curvature indicates the junction of the body and the pyloric part of the stomach. The greater curvature is convex and forms the left border of the stomach; it is about five times longer than the lesser curvature (Guyton and Hall, 2000).

Anatomically, the stomach is divided into four regions, cardia, fundus, body and pylorus (pyloric antrum). The cardiac part or cardia is an indefinite region around cardiac orifice which receives the opening of the abdominal part of the esophagus. The fundus is a dome-shape dilated portion to the left and superior to cardiac orifice, while the body forms the major portion of the left and superior to cardiac orifice, while the body forms the major portion of the stomach, it lies between the fundus and the pyloric antrum. The pyloric part consist of a wide portion the antrum and a narrow canal; the pyloric antrum is separated from the pyloric canal by an inconstant sulcus, sulcus intermedius that present on the greater curvature. It is about 7.5cm long. The pyloric canal is narrow and tubular about 2.5cm long at its right end it terminates at the pylorus. A distal sphincter region that guards the pyloric orifice separates the stomach from the duodenum (Guyton and Hall, 2000).

The known main physiological functions of the stomach are maintaining relatively low levels of microbes in the upper digestive tract, digestion of food and preparing dietary constituents for absorption, serving as a reservoir for swallowed food and drink and digestive secretions, mixing and delivery of the chyme to the small intestine at rates appropriate for further digestion and for absorption, and originating signals for hunger and satiety. It also acts as an endocrine organ to control its own function (Brooks, 1985).

In addition, the stomach must protect its mucosa from acid-pepsin digestion by maintaining a gastric mucosal barrier and from the enterogastric reflux of duodenal contents by reducing reflux as well as by maintaining an intact barrier. It also protects the oesophagel mucosa against reflux of acid-pepsin from the stomach by means of a gastroeosophageal sphincter mechanism (Brooks, 1985).

2.1.1 Blood Supply

The stomach has a rich arterial blood supply arising from the celiac trunk and its branches. Most blood is supplied by anastomoses formed along the lesser curvature by the right and left gastromental arteries. The fundus and upper body receive blood from the short and posterior gastric arteries. The gastric veins parallel the arteries in position and course, the right and left gastric veins drain into the portal vein. The short gastric vein and left gastromental vein empties in the superior mesenteric vein. A prepyloric vein ascends over the pylorus to the gastric vein (Moore and Dalley, 2006).

2.1.2 Nerve Supply

The gastrointestinal tract has a nervous system of its own called enteric nervous system (ENS). The ENS plays a crucial part in regulation of gastrointestinal function such as ion transport (secretion and absorption), motility and mucosal blood flow. As part of the autonomic nervous system, the ENS is organized in a complex but very sophisticated network. It lies entirely in the wall of the gut. The ENS is composed mainly of two plexuses; the myenteric or Auerbach’s plexus which lies between the longitudinal and circular muscle and mainly controls gastrointestinal movement. The submucosal or

Meissner's plexus that lies in the submucosa and controls secretion and blood flow (Guyton and Hall, 2006).

Sympathetic and parasympathetic fibres connect with ENS, although the ENS can function on its own independent of extrinsic nerve supply. Stimulation of the parasympathetic or sympathetic can further activate or inhibit gastrointestinal functions (Guyton and Hall, 2006).

The parasympathetic nerve fibres arise from the anterior and posterior vagal trunks and their branches. The anterior vagal trunk derived mainly from the left vagus (CN X), it supplies the lesser curvature of the stomach and gives rise to anterior gastric branches. The posterior vagal trunk, derived from right vagus nerve, supplies the anterior and posterior surface of the stomach. It gives off a celiac branch to the celiac plexus and continues along the lesser curvature as posterior gastric branches. Stimulation of parasympathetic nerve leads to contraction of the stomach wall, relaxation of the pyloric sphincter, secretion of HCl and pepsinogen and vasodilation of the gastric blood vessel. The sympathetic nerve supply arises from T6-T9 segment of the spinal cord and is distributed through the plexuses around the gastric and gastro-omental arteries. Its stimulation is the reverse case of parasympathetic nerve (Guyton and Hall, 2006).

2.2 ANATOMY OF THE RAT STOMACH

The stomach of albino rat consists of two portions, glandular and non-glandular or membranous portions. The two portions are separated from each other by the limiting ridge.

- The limiting ridge extends from the greater curvature to the lesser curvature. It begins at a point at the junction of the proximal (30%) and distal (70%) of the greater curvature and ends near the gastroeosophageal junction (Way, 1971).
- The non-glandular part (forestomach) is the whole area lying above the limiting ridge.The glandular part:The corpus an area which lies below the limiting ridge, and is full of gastric glands having zymogenic, parietal and mucous cells. This is comparable of the human stomach (Ahmed and Rao, 1975).
- The antrum is an area which is sharply delineated from the corpus by its lighter yellow colour and its thinner wall. This region is beset primarily with mucus glands (Robert and Nezamis, 1964).

2.3 FUNCTIONAL-ANATOMY OF THE GASTRODUODENAL MUCOSA

The mucosa consists of surface epithelia cells which are columnar in shape. This epithelium invaginate to varying extent into the underlying lamina propria glands to form gastric pits, which forms the opening of the gastric glands that lie in the lamina propria. The gastric glands are designated cardiac, gastric and pyloric glands, based on their anatomic position in the cardia, fundus and pyloric antral regions of the stomach. The gastric glands have characteristic parts which are the isthmus, the neck and the base. In the gastric glands, the isthmus contains surface epithelial cells and acidophilic parietal cells. The neck has the mucous neck cells and parietal cells, while the base is composed of basophilic zymogenic cells or chief cells with some differentiated cells. In the cardiac and pyloric glands, however, the different parts of the glands contain only one type of cell; they are tall columnar cells with mucigenic granules (Eroshenko, 1993).

The mucigenic cell, the mucus neck and the surface epithelial cells, all secrete a highly alkaline mucous secretion which is extruded to the surface to form a three-dimensional jelly coat for the surface of epithelial cells that are exposed to the digestive juices. The parietal cells of the fundic glands secrete hydrochloric acid (HCl) and intrinsic factor while the zymogenic cells of these glands produce the pepsinogen which in the presence of Hcl is activated to pepsin.Apart from the gastric glands, the lamina propria contains loose connective tissue interspaced with smooth muscle fibres and lymphoid cells that provide protection against bacterial infection. The deepest part of the mucosa is the muscularis mucosae which separate it from the subjacent submucosa, ulcerations sometimes gets to this layer and even beyond it and is accompanied by haemorrhages (Kumar and Clark, 1990).

The surface and pit cells are closely packed to one another forming tight junctions that form relatively impermeable interconnected sheaths that prevent back diffusion of hydrogen ions to the epithelial cells. These competent tight junctions were viewed as plugs that prevent diffusion of luminal contents through the paracellular pathways and into the interstitial fluid. The small size and numerous nature of these epithelial cells as well as the number of water filled channels limit transcellular diffusion through their surface protein-lipid apical cell membrane. The duodenal epithelial cells are tall columnar that are continuous with those of the glands of the lamina propria. These glands are called glands of lieberkuhn, the mucosa also consist of luminar projections about 0.5 – 1.5mm called villi. The duodenal mucosa have characteristic Brunner's gland that secrete a highly alkaline mucus secretions. This adaptive feature is to enable the region neutralizes the acidity and inactivates the enzyme pepsin present in the chyme reaching it from gastric emptying (Guyton, 1996).

2.3.1 Parietal or Oxyntic Cells

These cells are large, ovoid or polyhedral, with a large central nucleus. The unstimulated parietal cells have prominent cytoplasmic tubulovesicles and intracellular canaliculi containing short microvilli along its apical surface. Upon stimulation of cells this membrane along its apical surface transform into a dense network of apical intraceullular canaliculi containing long microvilli. An extensive eosinophilic (oxyntic) cytoplasm filled with mitochondria (30 to 40%) of total cell volume), generate energy required for secretion. H^+/K^+ ATPase is expressed in the tubulovesicle membrane while acid secretion, occurs at the apical canalicular surface. The enzyme H^+/K^+ ATPase is responsible for generating large concentration of H^+. It is a membrane-bound protein that consists of α and β subunits. The active catalytic site is found within α-subunit. This enzyme uses the chemical energy of ATP to transfer H^+ from parietal cell cytoplasm to secretory canaliculi in exchange for K^+. Parietal cells are the source of gastric HCl and in man intrinsic factor is also produced by the parietal cells. Intrinsic factor is a glycoprotein that binds ingested vitamin B_{12} and enables it to be absorbed by the intestine. A deficiency of intrinsic factor as a result of an autoimmune attack against parietal cells causes pernicious anemia (Del Valle, 2001).

The parietal cell expresses receptors for several stimulant of acid secretion including histamine (H_2), gastrin (gastrin-cholecystokinin B receptor) and acetylcholine (muscarinic, M_1). Each of these are G-protein linked seven transmembrane spanning receptors (Del Valle, 2001).

2.3.2 Chief Cells

Chief peptic or zymogen cells usually predominate in the lower portion of the fundal glands and towards the base of gastric glands. They are cuboidal or low columnar in shape with basally located nuclei. The upper two-third of the cells are filled with basophilic zymogene granules. The chief cells synthesize and secrete pepsinogen, the precursor to the proteolytic enzyme pepsin. Pepsin cleaves peptide bonds, favouring those on the C-terminal side of tyrosine, phenylalanine, and tryptophan residues. Its action breaks long polypeptide chains into shorter lengths. Secretion by the gastric glands is stimulated by the hormone gastrin. Gastrin is released by endocrine cells in the stomach in response to the arrival of food. Interspersed among the parietal and chief cells are enterochromaffin-like (ECL) and mass cells, which can stimulate acid and pepsinogen secretion by releasing histamine and peptides close to the exocrine cells (Del Valle, 2001).

2.3.3 Mucous Neck Cells

The mucous neck cells are found at the bottom of the gastric pits, they are regenerative cells which divide and replace the surface mucous cells as they move upward to the surface where they exfoliate. This process is rapid and averages five days in normal man, the rate probably increases when there is an injury to be repaired (Hotta, 2000). The population of cells at the fundal and pyloric gland areas is very stable and there replacement is probably accelerated only after injury. The mucigenic cells, the mucous neck cells and the surface epithelium cells all secrete a highly alkaline secretion which is extruded to the surface forming a three dimensional jelly coat for the surface epithelial cells that are exposed to digestive juices (Del Valle, 2001).

2.3.4 Hormone-Secreting (Endocrine) Cells

Near the basal part of gastric gland are endocrine cells that contain membrane bond neurosecretory granules. These cells secrete the hormone gastrin and serotonin (5HT) the stomach releases two hormones into the blood; gastrin and somatostatin. Serotonin staining (ECL) cells are also present in the gastric fundus and antrum. Gastrin stimulates acid secretion by the stomach and somatostatin inhibits the release of gastrin (Guyton and Hall, 2000).

2.4 GASTRIC SECRETION

The fluid secreted into the stomach is called gastric juice. It is a mixture of secretions of the epithelial cells and gastric glands situated in the mucosal lining of the stomach. Gastric secretory products include salt, water, HCL, pepsinogen, intrinsic factor and electrolytes (Guyton and Hall, 2000). The best known component of gastric juice is hydrochloric acid, the secretory product of the parietal, or oxyntic cell (Yao and Forte, 2003). However, hydrochloric acid and pepsinogen are the main products capable of inducing mucosal damage (Guyton and Hall, 2000).

2.4.1 Mechanism of Gastric Acid Secretion

The purest specimen of parietal cells secretion that have been obtained contained approximately 0.17µHcl with pH as low as 0.87, but the acid acts diluted in the stomach lumen to the pH between 2 and 3 (Wikipedia, 2006).

When the parietal cell is stimulated, it secretes an acid solution that contains about 160mm of HCl/litres, which is almost exactly isotonic with the body fluid. In the parietal cells the carbon dioxide m (CO_2) combined with water to form carbonic acid (H_2CO_3). CO_2 is derived by metabolic activity of the cells. Some amount of CO_2 is obtained from blood. The carbonic acid is formed in the presence of the enzymes carbonic anhydrase which is present in high concentrations in the parietal cells (Sembulingam and Sembulingam, 2003). The H_2CO_3 acid formed will dissociate to form H^+ and HCO^-_3. The HCO^-_3 diffuse into the blood in exchange with chloride ions (Abu-Sitta *et al*., 2000). Water becomes dissociated into hydrogen ion (H^+) and hydroxyl ion (OH^-) in the cell cytoplasm. The H^+ are then actively secreted into the canaliculi in exchanged for potassium ions. This process of active exchange is catalyzed by H^+/K^+ ATPase (Guyton and Hall, 2002). OH^- will combine with the H^+ from carbonic acid to form water (Abu-Sitta *et al.*, 2000). Chloride ion (Cl^-) is also pumped into the canaliculi actively (down an electrochemical gradient). The Cl^- is derived from NaCl of blood. Now the H^+ will combine with the Cl^- to form HCl (Sembulingam, 2003).

Usually gastric acid secretion is in three phases, viz – the cephalic phase under the influence of vagal discharges, is secreted in response to the smell, thought or taste of palatable food (Ganong, 1997). The gastric phase which is induced by the actual presence of food in the stomach and the intestinal phase induced by the presence of product of gastric digestion in the duodenum (Guyton, 1996).

2.4.2 Mechanism of Hydrochloric Acid Secretion

Hydrochloric acid is an important known component of gastric juice, secreted from parietal, or oxyntic cell. It is known that the capacity of the stomach to secrete HCl is almost linearly related to parietal cell numbers. When stimulated, parietal cells secrete HCL at a concentration of roughly 160 mM (equivalent to a pH of 0.8). The acid is secreted into large canaliculi, deep invaginations of the plasma membrane which are continuous with the lumen of the stomach. Upon stimulation of acid secretion, dramatic changes exist in the morphology of the membranes of the parietal cell. Cytoplasmic tubulovesicular membranes which are abundant in the resting cell virtually disappear in concert with a large increase in the membrane. The proton pump as well as potassium and chloride conductance channels initially reside on intracellular membranes and are transported to and fused into the membrane just prior to acid secretion. The hydrogen ion concentration in parietal cell secretions is roughly 3 million fold higher than in blood and chloride is secreted against both a concentration and electric gradient. Thus, the ability of the parietal cell to secrete acid is dependent on active transport and the key player in acid secretion is an H^+/K^+ ATPase or "proton pump" located in the membrane K^+ ATPase are enzymes responsible for generating the large concentration of H^+. The enzyme uses the chemical energy of ATP to transfer H^+ ions from parietal cell cytoplasm to the secretory canaliculi in exchange for K^+; this ATpase is magnesium-dependent, and not inhibitable by ouabain (Yao and Forte, 2003). H^+/K^+ ATPase is located within the secretory canaliculus and in non secretory cytoplasmic tubulovesicles. The tubulovesicles are impermeable to K^+ which leads to an inactive pump in this location. The distribution of pumps between the nonsecretory vesicles and secretory canaliculus varies with parietal cell activity. Under resting conditions, only 5% of the pumps are within the secretory canaliculus, whereas upon parietal cell stimulation, tubulovesicles are immediately transferred to secretory canalicular membrane, where 60 to 70% of the pumps are activated (Del Valle, 2001).

2.4.3 Pepsinogen Secretion

The chief cells in gastric glands as well as mucous cells secrete pepsinogens, a group of proteolytic proenzymes (zymogens or inactive enzyme precursors) that belong to the general class of aspartic proteinases. Pepsinogen becomes activated to form active pepsin in the presence of hydrochloric acid. pepsinogen secretion in the basal state is approximately 20% of its maximal secretion after stimulation. Although pepsinogen secretion generally parallel that of acid secretion, but the cellular mechanism of pepsinogen secretion is quite distinct that of H^+ secretion by parietal cells (Boron and Boulpaep, 2005).

The regulation of pepsinogen secretion occurs in response to two types of signals: stimulation of the peptic cells by acetylcholine released from the vagus nerve or from gastric enteric nervous plexus and stimulation of peptic secretion in response to acid in the stomach. The acid may not stimulate the peptic cells directly but elicits enteric reflexes, thus supporting the original nervous signals to the peptic cells (Del Valle, 2001).

Release of pepsinogen across the apical membrane results from a process of compound exocytosis. This process permits rapid and sustained secretion of pepsinogen. After stimulation the initial peak in pepsinogen secretion is followed by a persistent lower rate of secretion. Two groups of agonists stimulate chief cells to secrete pepsinogen. One group acts through adenylyl cyclase and cAMP, and the other acts through increase in Ca^+. Chief cells have receptors for secretin/VIP, β_2-adrenergic receptor, and receptor for PGE_2 which activate adenylyl cylase. While M_3 receptor for acetylcholine, as well as receptors for gastrin/CCK family of peptides acts through increases in Ca^+. Unlike gastric acid secretion which is stimulated via CCK_9 receptor pepsinogen secretion is stimulated via the CCK receptor. However, the most important agonist for pepsinogen secretion is acetylcholine release in response to vagal stimulation (Boron and Boulpaep, 2005).

2.4.4 Feedback Inhibition of Gastric Acid Secretion

When the acidity of the gastric juice increases to a pH of 2.0, the gastrin mechanism for stimulating gastric secretion becomes totally blocked. This effect probably results from two different factors. First, greatly enhanced acidity depresses or blocks the extraction of gastrin itself from the antral mucosa. Second, the acid seems to cause an inhibitory nervous reflex that inhibits gastric acid secretion. This feedback inhibition of the gastric glands plays an important role in protecting the stomach against excessively acid secretions, which would readily cause peptic ulceration. Also, the feedback mechanism is important in maintaining optimal pH for function of the peptic enzymes in the digestive process, where gastrin begins to be secreted again and more acid is secreted when the pH rises above 2.5 to 3.5 (Guyton, 1996).

2.4.5 Inhibition of Gastric Secretion by Intestinal Factors

Though chyme stimulates gastric secretion during the intestinal phase of secretion. It paradoxically often inhibits secretion during the gastric phase. This results from at least two factors:

i) The presence of food in the small intestine initiates an enterogastric reflex, transmitted through the enteric nervous system as well as the extrinsic sympathetic and vagus nerves that inhibits stomach secretion. This reflex can be initiated by distension of the small bowel, the presence of acid in the upper intestine, the presence of fats and carbohydrates or irritation of the mucosa. This is part of the complex mechanism for slowing down stomach emptying when the intestines are already filled.

ii) The presence of acid, fat, protein breakdown products, hyper–or hypo–osmotic fluids, or any irritating factor in the upper small intestine causes the release of several intestinal hormones. Two of these are secretin and cholecystokinin (CCK). Both are specially important for control of pancreatic secretion, and CCK is also important for emptying of the gall bladder. In addition, the previous two hormones oppose the stimulatory effects of gastrin. Also, three other hormones – gastric inhibitory peptide (GIP), VIP and somatostatin – all have slight to moderate effects on inhibiting gastric secretion. In fact, it was found that the enterogastric reflex and these inhibitory hormones usually reduce stomach motility at the same time that they reduce gastric secretion (Guyton, 1996).

2.4.6 Regulation of Gastric Acid Secretion

This occurs primarily in three phases: cephalic phase stimulates gastric secretion via the vagus nerve, impulses results from the sight, smell and taste of food. the gastric phase is activated once food enters the stomach. This component of secretion is driven by mechanical distention of the stomach and nutrients (amino acids and amines) that directly stimulate G-cells to release gastrin, which in turn activates the parietal cell via direct and indirect mechanisms. The third phase of gastric acid secretion is initiated as food enters the intestine and is mediated by luminal distention and nutrient assimilation. Additional neural (central or peripheral) and direct hormonal (secretin, cholecystokinin etc) factors, play a role in counterbalancing acid secretion (Del Valle, 2001).

Stimulation of Parietal Cell

All signals converge on the gastric parietal cell located on oxyntic gland, on several stimulant of acid secretion. The cephalic-vagal or gastric-vagal efferents directly stimulate the parietal cell via the muscarinic (M_3) type of receptors for acetylcholine while gastrin apparently activates a gastrin-cholecystokinin B receptor (CCKB). A cell designated the enterochromaffin-like cells plays a central role. Gastric and vagal efferents induce the release of histamine from the enterochromaffin-like cell, thereby stimulating the histamine (H_2) receptor on parietal cells. Binding of histamine to H_2 receptor leads to activation of adenylate cyclase and an increase in cyclic AMP. Activation of the gastrin and muscarinic receptors results in activation of the protein kinase C/phosphoinositide signaling pathway. Each of these signaling pathways in turn regulates a series of kinase cascades, which control the acid-secreting pump, H^+, K^+ ATpase. The discovery that different ligands and their corresponding receptors lead to activation of different signaling pathways explains the potention of acid secretion that occurs when histamine and gastrin or acetylcholine are combined. Parietal cells also secrete intrinsic factor and express receptors for ligands that inhibit acid production (prostaglandins, somatostatin and EGF) (Del Valle, 2001). Somatostatin, release by gastric D cells in the antral and corpus of the stomach, is the central mechanism of inhibition of acid secretion, however, several enteric hormones (Enterogastrone) and prostaglandins inhibit gastric-acid secretion through a negative feedback mechanism (Boron and Boulpaep, 2005).

2.5 PEPTIC ULCER DISEASE (PUD)

Peptic ulcers are breakages in the continuity of the mucosal epithelial lining of the stomach, duodenum and at times lower oesophagus and Merkel's diverticulum. It can also occur in areas of ectopic gastric tissue, and in small and large bowels in Zollinger-Ellison syndrome (Falase and Akinkugbe, 2000).

It is also a chronic inflammatory condition of the stomach and duodenum that affects more than 4 million people each year in the United States (Graham, 1996). It is a significant public health problem that exerts a high cost to the society (Vaket and Fennerly, 1996). Gastric hyperacidity and ulcer are very common causing human suffering today (Rao *et al.*, 2000).

Gastric ulcers are caused due to imbalances between offensive and defensive factors of the gastric mucosa. The anti-ulcerogenic activity of the many plant products is reported due to an increase in mucosal defensive factors rather than decrease in the offensive factors (Goel and Chakrabathy, 1985).

Until about a decade ago, the pathogenesis of this disorder was believed to be related to stress, gastric acid hypersecretion and other related factors. As such treatment include antacids, H_2–receptor antagonists, and even bland foods. The discovery in 1983 by Warren and Marshall of a gram-negative, spiral shaped, microaerophilic bacterium identified as *helicobacter pylori*, dramatically changed the management of peptic ulcer disease (Falk, 2000).

Although prolonged anxiety, emotional stress, haemorrhagic 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 irritation/ulceration in human and experimental animals (Rao *et al.,* 2000).

Most of the available drugs are thought to act on the offensive factors which neutralize acid secretion like antacids, H_2-receptor blockers like ranitidine, famotidine, anticholinergics like pirenzepine, telezipine, proton pump inhibitors like omeprazole lansoprazole etc which interfere with acid secretion. Recently, the involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration (Sairamet *et al.,* 2001).

In the absence of non-steroidal anti-inflammatory drugs (NSAIDs) use or acid hypersecretory states such as gastrinomas, helicobacter pylori has now been found to be the causative factor in 75% of all patients with gastric ulcer and 90% of all patients with duodenal ulcer (Falk, 2000).

The mechanism of helicobacter pylori-related inflammation and ulcer formation is not fully understood. However, significance rates and ulcer healing with antibiotic therapy confirms the significance of helicobacter pylori in peptic ulcer disorder (Suebauerm and Michetti, 2002).

2.5.1 Pathophysiology of Peptic Ulcer Disease

It is now generally agreed that gastric lesions develop when the delicate balance between some gastroprotective and aggressive factors is lost. The major aggressive factors are acid, pepsin, helicobacter pylori and bite salts. Defensive factors mainly involve mucous-bicarbonate secretion and prostaglandins (Hoogerwerf and Pasricha, 2001).

Helicobacter Pylori

Of recent, infection with the bacterium helicobacter pylori is proven to play a major part in the development of peptic ulcer, particularly duedenal ulcers, and its successful eradication often lead to cure of peptic ulcer disease (Falase ad Akinkugbe, 2000).

It is a spirochaectal bacterium that exists in the duodenum deep to the mucosal layer (Bailey and Loves, 1992). About 90% of endoscopically proven ulcers are as a result of infection with helicobacter pylori. So significant is the connection with ulcers, helicobacter pylori infection and subsequent gastric malignancies that the World Health Organization has classified helicobacter pylori as a class 1 carcinogen (Huang *et al*., 2002 and NIH, 2002).

One of the key survival mechanisms of helicobacter pylori in the acidic gastric environment is the urease activity (Decross and Marshall, 1993). It has the ability to split urea with the formation of ammonia. The consequential local (and sometimes extreme) rise in pH has been blamed for causing epithelial cellular damage and ulceration (Bailey and Loves, 1992). Urease is a surface bond enzyme that cleaves urea to form ammonia and carbon dioxide, making the environment surrounding helicobacter pylori relatively alkaline (Decross and Marshall, 1993). This permits the bacterium to buffer its micro-environment and facilitate its survival when exposed to low pH in the gastric lumen (Falk, 2000).

Most urease is found in the bacterium cytoplasm. Helicobacter pylori expresses a unique protein called urel that serves as an H^+ gated channel. Urea transport through urel allows helicobacter pylori to survive in the acidic environment of the stomach. Initiation of urel prevents helicobacter pylori urease activity, presenting a means of eradicating the organism in the stomach acidity without the use of antibodies (Weeks *et al.,* 2000). Certainly, if the organism is removed by antibiotics (metronidazole) or bismuth salts (tripotassium dicitratobismuthate), the recurrent ulcer rate is reduced (Bailey and Loves, 1992).

Apoptosis also occurs because of nitric oxide production through induction of nitric oxide synthase by helicobacter pylori. Involvement of reactive oxygen species (ROS) and oxidative damage of DNA and DNA fragmentation have also been evident in cell death in gastric mucosal injury by *H. pylori* (Konturek *et al.*, 1999).

Additionally, *H. pylori* produces protease, lipase and phospholipases which hydrolyze the surface mucous layer and facilitate penetration into the mucous coat (Huang *et al.,* 2002). Since gastric mucosal integrity is maintained by a dynamic process of cell death and cell proliferation in which apoptotic cell death play significant roles in the loss of gastric mucosal integrity, the role of *H. pylori* in these complex mechanisms cannot be over-emphasized (Das *et al.,* 1997).

When infected by *H. pylori* within few days, patient develop dyspepsia, epigastric pain, nausea and at times with vomitting. Shortly thereafter, there is a period of hypochlorrhydria that persists for few months, after which the intragastric pH returns to normal levels (Rave, 1999). This transient reduction in activity could weaken the first line of defence against pathogenic organisms and could make the host more susceptible to enteric infections (Falk, 2000).

At the early phase of the infection there is massive infiltration of neutrophil granulocytes and production of antibodies of IgA, IgG and IgM (Rave, 1999). However, these do not eradicate *H. pylori*. The organism is able to induce the release of interleukin 8(Bourke *et al.,* 1996) from the epithelium. This is chemotactic to neutrophils that invade the superficial layer of the gastric mucosa and thereby help sustain the infection (Falk, 2000).

Helicobacter pylori infection leads to gastroduedonal inflammation, peptic ulceration, gastric lymphoma, gastric cancer which has been proved with animal studies and human epidemiological reports (Forman *et al.,* 1996; Negrini *et al.,* 1996; Fan *et al.,* 1996; Parsonnet *et al*., 1991).

Helicobacter pylori may induce inflammatory associated gene expression in gastric epithelial cells, including activation of nuclear factor kapper B (MF-KB), enhance expression of cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) and production of interleukin 8 (Il-8) (Kim *et al.,* 2001; Guo *et al.*, 2003). NF-KB activation accompanied by a decrease in IKBX in helicobacter pylori-infected AGS cells (Chu *et al.,* 2003). These genes are important factors of gastric inflammation.

It is widely accepted that most peptic ulcers are associated with helicobacter pylori infection and eradication of the organism leads to enhanced ulcer healing and reduced the chance of ulcer recurrence (Soll, 1996).

Helicobacter pylori may cause NF-kB activation and iNOS expression, which induces high nitric oxide (NO) production and apoptosis in gastric epithelial cells (Lim *et al,* 2001).

Research has indicated that there may be an immune response that has a role in ulcer formation (CDC, 2002). Eradication of, helicobacter pylori is being considered nor by many gastroenterologists as the first line goal of therapy (Chimize, 2001).

Research has also shown that helicobacter pylori infection increases the incidence of peptic ulcer disease among NSAID users by 3:1, and increased the risk for ulcer by 6:1 (Huang, 2002). This is in sharp contrast to the restrictive dietary constraint and dependence on medication previously necessary for ulcer management. Efficiency of a vaccine for the *H. pylori* is currently under study (NIH, 2002).

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDS) such as indomethacin, aspirin and piroxicam, remain among the most commonly used pharmacological agents (Garner, 1992). However, these classes of substances may cause gastrointestinal ulceration, due to ability of these agents to suppress prostaglandin synthesis (Wallace, 2000).

Piroxicam is a preferential COX-1 inhibitor. Cyclooxygenase was constitutively expressed in the gastrointestinal tract in large amounts and has been reported to maintain mucosal integrity through continuous generation of prostaglandins (Halter *et al.*, 2000).

NSAIDs are worldwide used for the treatment of pain, rheumatic and cardiovascular diseases and more recently for the prevention of colon cancer and Alzheimer's disease (To *et al.*, 2001).

The mechanism by which NSAIDs cause injury to the gastric mucosa is mainly due to the inhibition of cyclooxygenase enzyme (COX) production and suppression of prostaglandin (PG) mediated effects on mucosal protection (Wallace *et al.*, 2000). Besides, it has been proposed that neutrophil and oxygen radical dependent microvascular injuries may be important processes that lead to mucosal damage in response to NSAIDs administration (Wallace and Granger, 1992). These agents cause the activation of neutrophils and their adherence to the vascular endothelium, hence blocking capillaries and reducing local gastric blood flow.

Indomethacin

Indomethacin, a potent anti-inflammatory drug (NSAID), was introduced in 1963 for the treatment of rheumatoid arthritis and related diseases. Eight years later, it was demonstrated that indomethacin and aspirin inhibit prostaglandin (PG) biosynthesis (Ferreira *et al*., 1971; Vane, 1971). A reduction in the biosynthesis of PG through inhibition of cyclooxygenase (COX) is the pharmacological background to the anti-inflammatory action (Vane, 1971) and the harmful side effects of indomethacin, as well as other NSAIDs (Hawkey, 2000). The gastrointestinal side effects of NSAIDs, especially in the stomach, are one of the more serious complications in patients taking these drugs (Hawkey, 2000; Wallace, 1997). Indeed, indomethacin shows potent ulcerogenic action in experimental animals (Takeuchi *et al*., 1986; Wallace, 1997). The mechanism by which indomethacin induces gastric injury is generally considered to involve depletion of PGs, yet it has proven more complicated than expected and involves multiple, closely interacting elements such as gastric hypermotility, microcirculatory disturbances, neutrophil-endothelial cell interactions, and superoxide radicals, in addition to PG deficiency (Takeuchi *et al*., 1991; Wallace, 1997).

Most of the available gastroprotective drugs act on the offensive factors neutralizing acid secretion like antacids, H_2-receptor blockers like ranitidine, anti-chlolinergics like pirenzepine, proton pump inhibitors like omeprazole, lansoprazole etc which interfere with acid secretion (Rao *et al.,* 2004).

Synthetic NSAIDs like aspirin cause mucosal damage by intefering with prostaglandin synthesis increasing acid secretion and back diffusion of H^+ ions, resulting in overproduction of leukotrienes and other products of 5-lipooxygenase pathway (Rao *et al.,* 2000).

Reactive oxygen species, especially the hydroxyl radicals, play a major role in oxidative damage of gastric mucosa in all forms of gastric ulcer (Das *et al.,* 1997; Phull *et al.,* 1995).

Gastroduodenal ulceration is a common disease in both developed and developing countries (Pavo *et al.,* 2000). Deficiency of endogenous prostaglandins is widely accepted as a major factor in the pathogenesis of gastric lesions caused by NSAIDs. Prostaglandins (PGs), especially PGE_2 and their analogues inhibit the formation of gastric mucosal necrosis induced by necrotizing agents. This protective action of PGs against the direct injuries of gastric necrotizing agents is apparently unrelated to their antisecretory properties and has generally been called cytoprotection (Peskar and Maricic, 1998).

A large number of anti-inflammatory agents show a tendency to produce gastric irration, bleeding and mucosal cellular damage (Shiotani and Graham, 2002).

Although hospital admission for uncomplicated peptic ulcer in developed countries had began to decrease, there was a striking rise in admissions for, ulcer haemorrhage and perforation among elderly people (Higham *et al.,* 2002). This increase has been attributed to the increased use of non-steroidal anti-inflammatory drugs (NSAIDs), alcoholic beverages, cigarettes and *Helicobacter pylori* infections (O'Malley, 2003).

Borrelli and Izzo (2000) reveal the extensive variety of chemical compounds isolated from medicinal plants with antiulcer activity. This is an important reason to investigate antiulcer effects in medicinal plants with traditional use in gastric diseases. This indicates their great potential in the discovery of new therapies for ulcers.

In this way, the peptic ulcer is considered a disease of modern times, related to the addictions that are increasingly frequent in the society and to its stressful lifestyle. Treatment with natural products presents promise of a cure. Plants have been raw material for the synthesis of many drugs, and they remain an important source of new therapeutic agents.

Upon chronic consumption of NSAIDS during the course of anti-inflammatory therapy, prostaglandin synthase enzyme is inhibited through the cyclooxygenase pathway (Rainsford, 1987). In the stomach prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair (Hayllar and Bjarnason, 1995). Thus, the suppression of prostaglandin synthesis by NSAIDS results in increased susceptibility to mucosal injury and gastroduedenal ulceration.

Cimetidine

Cimetidine is a histamine H_2-receptor antagonist. It reduces basal and nocturnal gastric acid secretion and a reduction in gastric volume, acidity, and amount of gastric acid released in response to stimuli including food, caffeine, insulin, betazole, or pentagastrin. It is used to treat gastrointestinal disorders such as gastric or duodenal ulcer, gastroesophageal reflux disease, and pathological hypersecretory conditions. Cimetidine inhibits many of the isoenzymes of the hepatic CYP450 enzyme system. Other actions of Cimetidine include an increase in gastric bacterial flora such as nitrate-reducing organisms. For the treatment and the management of acid-reflux disorders (GERD), peptic ulcer disease, heartburn, and acid indigestion.

Cimetidine binds to an H_2-receptor located on the basolateral membrane of the gastric parietal cell, blocking histamine effects. This competitive inhibition results in reduced gastric acid secretion and a reduction in gastric volume and acidity.

Other Contributing Factors to Peptic Ulcer Disease

Helicobacter pylori infection or NSAID alone may not be sufficient to cause peptic ulcer disease. Genetic and environmental factors may also contribute. For example, people with duodenal ulcers are more likely to have fairly members with duodenal ulcers compared to the general population. Another factor is cigarette smoking which increases the risk of developing ulcers, and impairs healing. Alcohol abuse also appears to interfere with ulcer healing (Fries, 1991).

Alcohol

Ethanol induces gastric mucosal damage due to a direct action on the gastric epithelium causing lipid peroxidation mediated by interaction of hydroxyl radicals with the cell membrane; subsequently producing lipid-derived radicals that are extremely reactive products causing oxidative damage (Repetto and Liesuy, 2002).

In a study by Bagchi et *al*. (1998) reported that ethanol treatment Increases superoxide anion, hydroxyl radical production and lipid peroxidation in the gastric mucosa. Similarly acute ethanol treatment induces oxidative stress, DNA damage, increased xanthine oxidase activity, and decreased total ESH content in the gastric mucosal cells (Gonzalez *et al.,* 2001). A study that examines alterations of gastric ulcer and healing responses by Kato *et al.,* (2000) reported that unlcerogenic response to ethanol was prevented by inducible nitric oxide synthase and was also partially related to elevated production of prostalglandin and cyclooxygenase.

Antioxidants

These are compounds that guard and protect the cells against the damaging effects of reactive oxygen species such as superoxide, peroxyl radicals, hydroxyl radicals, peroxynitrite and singlet oxygen. An imbalance between antioxidants and reactive oxygen species results in oxidative stress leading to cellular damage (Burker *et al.,* 2003). They also inhibit other free radical mediated processes protecting the human body from several diseases attributed to the reaction of radicals (Czinner *et al.,* 2001).

Their molecules are known to neutralize free radicals by accepting or donating an electron to eliminate the impaired condition. This means that the antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a non-free radical molecule. But the antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized. It may be readily neutralized by another antioxidant and/or it may have another mechanism for terminating its free radical condition. Molecules with loosely held hydrogen atoms can use those hydrogen atoms like electrons to neutralize free radicals. The hydrogen atoms are called reducing equivalents, and the molecules having such hydrogen atoms are said to be in a reduced state (Wei, 2002).

Antioxidants are also claimed to inhibit lipid peroxidation and scavenge free radicals (Di Carlo *et al.,* 1999). Studies by Alanko, 1999 have demonstrated that flavonoids found in fruits and vegetables may also act as antioxidants, in that they contain chemical structural elements that may be responsible for their antioxidant activities. However, the recognized dietary antioxidants are vitamin C, vitamin E., caroteroids and selenium.

Also, natural antioxidant enzymes manufactured in the body provide an important defence against free radicals. Glutathione peroxidise, glutathione reductase, catalase, thioredoxin reductase and superoxide dismutase are the important antioxidant enzymes (Marklund, 1982).

Lipid Peroxidation

The process of lipid peroxidation is mediated by the interaction of hydroxyl radicals with the cell membrane; subsequently producing lipid derived free radicals such as conjugated dimes and lipid hydroperoxides (Repetto and Liesuy, 2002). These radicals are known to be extremely reactive products that cause oxidative damage.

2.5.2 Diagnosis of Peptic Ulcer Disease

Diagnosis of peptic ulcer is difficult to establish with symptoms alone and it is in fact difficult to differentiate between duodenal and gastric ulcers based on symptoms alone. The importance of investigation is therefore emphasized in making a complex distinction and therefore a definitive diagnosis (Davidson, 1995).

Laboratory Studies

In most patients with uncomplicated peptic ulcer disease, routine laboratory tests usually are not helpful. Diagnosis of peptic ulcer disease depends on radiographic and endoscopic confirmation. If the diagnosis of peptic ulcer disease is unclear or complicated, peptic ulcer disease is suspected, liver function test (LFTs), amylase, and lipase might be useful (Yamada *et al*., 1995).

Imaging Studies

Double-contrast radiography, performed by an experienced radiologist might approach a diagnostic accuracy of upper gastrointestinal endoscopy. However, it is not as sensitive as endoscopy for establishing a diagnosis of small ulcer (<0.05cm).It also does not allow for obtaining a biopsy to rule out malignancy in the setting of a gastric ulcer or to assess for *H. pylori* infection in the setting of a gastroduodenal ulcer (Gisbert *et al*., 2004).

Other Tests

Detection of H. pylori infection is essential in all patients with peptic ulcer. Endoscopic or invasive tests include a rapid urease test, histopathology and culture. Rapid urease test and endoscopy are diagnostic tests of choice. The presence of *H. pylori* in gastric mucosal biopsy specimens is detected by testing for bacterial urease. Three kits (i.e. clotest®, Hp-fast®, pylori Test®) are commercially available, each containing combination of a urea substrate and a pH sensitive indicator. One or more gastric biopsy specimens are placed in the rapid urease test kit. If *H. pylori* is present, bacterial urease convert urea to ammonia, which changes pH and produces a colour change obtained histopathology, often considered the criteria standard to establish a diagnosis of *H. pylori* infection. If the rapid urease test result is negative and a high suspicion for *H. pylori* persist (presence of a duodenal ulcer) (Gisbert *et al*, 2004). Culture is used primarily in research studies and is not available routinely for clinical use. Non-endoscopic or non-invasive tests include serum *H. pylori* antibody detection, faecal antigen test, and urea breath tests. Antibodies (immunoglobnulin G [IgG]) to *H. pylori* can be measured in serum, plasma, or whole blood. Result with whole blood test obtained from finger sticks are less reliable (Rautelin *et al*., 2003).

Urea breath test detect active *H. pylori* infection by testing for the enzymatic activity of bacterial urease. In the presence of urea produced by *H. pylori*, labeled carbon dioxide (heavy isotope, carbon 13, or radioactive isotope, carbon 14) is produced in the stomach, absorbed into the blood stream, diffused into the lungs and exhaled (Yamada *et al*., 1995).

Faecal antigen testing identifies active *H. pylori* infection by detecting the presence of *H. pylori* antigens in stool. This test is more accurate than antibody testing and is less expensive than urea breath test (Rautelin *et al*., 2003).

Special Studies

A fasting serum gastrin level should be obtained in certain cases to screen for Zollinger-Ellison syndrome such cases include the following: patient with multiple ulcers; ulcer occurring distal to the duodenal bulb; strong family history of peptic ulcer disease; peptic ulcer not associated with H. pylori infection or NSAIDS use; peptic ulcer associated with diarrhea, steatorrhea; or weight loss; peptic ulcer associated with hypercalcemia or renal stones; ulcer refractory to medical therapy; and ulcer recurrence after surgery (Hughes, 1993).

A secretin stimulating test may be used if the diagnosis of Zollinger-Ellison syndrome cannot be made with the gastrin level alone. This test can distinguish Zollinger-Ellison syndrome from other conditions with high serum gastrin level such as antisecretory therapy with a proton-pump inhibitor, renal failure, or gastric outlet obstruction (Hughes, 1993).

Upper Gastrointestinal Endoscopy

This is the preferred diagnostic test in the evaluation of patients with suspected peptic ulcer disease. Highly sensitive for the diagnosis of gastric and duodenal ulcers. Allows for biopsies and cytologic brushing in the setting of a gastric ulcer to differentiate a benign ulcer form a malignant lesions. Allow for detection of H. pylori infection with antral biopsies for a rapid urease test and/or histopathology in patients with peptic ulcer disease (Hughes, 1993).

2.5.3 Management of Peptic Ulcer

Prior to the discovery of the H_2 receptor antagonists and the realization of the role of *H. pylori* infection in the development of peptic ulcers, therapy consists of antacids, anticholinergics and a bland diet. The introduction of antisecretory drugs, H_2 receptor antagonists and proton pump inhibitors, along with the utilization of ,upper gastrointestinal gastroscopy and the development of antibiotic combination therapies for eradication of *H. pylori* have revolutionized peptic ulcer therapy and results not only in healing the ulcer, but also in curing many cases (Sharma and Ahuja, 2003).

Physiological Basis of Management

The aim of management is to relieve symptoms and induce healing of ulcer in short-term and prevent relapse in the long-term (Davidson, 1995). Management would therefore include general measures that would aid the physiological mechanisms help heal the body, drug therapy and surgical therapy if need be.

General Measures

General measures include discontinuation of NSAIDs, discourage smoking since it confers risk and prevent normal healing in response to the normal drug regimen. Alcohol in moderation is not harmful but may be totally discouraged (Davidson, 1995). It is usually recommended that the patient eats frequent small meals consisting of food known to but non-irritating. For many, but not all patients, fatty, highly spiced, heavy or fibrous foods are likely to provoke pain (Anderson *et al.*, 1994). Although it is also documented that no special dietary advice is required since more recently the importance of diet in therapy has been minimized (Davidson, 1995).

2.5.4 Drug Management

Acid suppression is the general pharmacologic principle of medical management of acute bleeding from a peptic ulcer. Reducing gastric acidity is believed to improve haemostasis, primarily during the decreased activity of pepsin in the presence of more alkaline environment. Two classes of acid-suppressing medications currently in use are H_2RAS (H_2 receptor antagonists and proton-pump inhibitors [PPI] (Nerstard, 1997). Example of H_2RAS includes ranitidine, cimetidine famotidine and nizatidine. Examples of proton-pump inhibitors include omeprazole, pantoprazole, lansoprazole and rabeprazole (Soll, 1996).

Many gastroenterologists assert that intravenous proton-pump inhibitor therapy maintain haemostasis more effectively than intravenous H_2RAS. Many researchers have compared parenteral proton-pump inhibitor therapy to placebo. Overall, the results demonstrate shorter bleeding and demonstrated decrease need for emergency surgery and blood transfusion.

Concomitant *H. pylori* infection in the setting of bleeding peptic ulcer should be eradicated as this lowers the rate of bleeding. The goals of pharmacotherapy are to eradicate *H. pylori* infection to reduce morbidity and to prevent complications (Kikkawa *et al*., 2005).

Proton Pump Inhibitors

Inhibitors of the gastric H^+/K^+ ATpase (proton-pump) enzyme system which catalyzed the exchange of H^+ and K^+ (Gisbert, 2005).

(a) Omeprazole (prilosec)

Suppresses gastric acid secretion by specifically inhibiting H^+/K^+ ATpase system at secretory surface of gastric parietal cells.

Adult dose for *H. pylori* eradication	20mg po bid
Uncomplicated duodenal ulcer	20mg po qid
Refractory duodenal ulcer	40mg po qid

(Laujy *et al*., 2000)

(b) Pantoprazole (protonix, pantoloc)

Suppresses gastric acid secretion by specifically inhibiting H^+/K^+ ATpase system at secretory surface of gastric parietal cells. Indicative for <8 weeks treatment of erosive esophagitis associated with GERD. May consider additional 8-wks course if ulcer does not heal.

Adult dose 40mg po qid (Mertz *et al.*, 2000)

(c) Lansoprazole (prevacid)

Suppresses gastric acid secretion by specifically inhibiting H^+/K^+ ATpase system at secretory surface of gastric parietal cells.

Used for <4 weeks to treat and relieve symptoms of peptic ulcer.

Adult dose for *H. pylori* eradication 30mg po qid

Maintenance 15mg po qid

(Maiden *et al.*, 2000)

(d) Esomeprazole magnesium (Nexium)

Suppress gastric acid secretion by specifically inhibiting H^+/K^+ ATPase system at secretory surface of gastric parietal cells.

Adult dose 20-40mg po qd for 4-8 weeks (Lin *et al.*, 1998)

Antihistamine, H_2 Receptor Blockers

These agents are used in the short-term treatment of active duodenal ulcer or gastric ulcer and long-term as prophylaxis (Netzer *et al.*, 1999).

(a) Famotidine (Pepcid AC, Pepcid)

Competitively inhibit histamine at H_2 receptor of gastric parietal cells reducing gastric acid secretion, gastric secretory volume and hydrogen concentration.

Adult dose initial 40mg po hs

Maintenance 20mg po bid

(Soll, 1996)

(b) Nizatidine (Axid)

Competitively inhibit histamine at H_2 receptor of gastric parietal cells reducing gastric acid secretions.

Adult dose initial 300mg po or 150mg bid

Maintenance 50mg po qid

(Netzer *et al.*, 1999)

(c) Ranitidine bismuth citrate (Tritec, pylorid)

Combination of ranitidine and bismuth citrate compound with bacteriocidal effect against *H. pylori* used with clarithromycin because drugs act synergistically against *H. pylori*.

Adult dose 400mg po qid for 14 days for *H. pylori* eradication (Merk, 1994)

(d) Cimetidine (Tagamet, peptol)

Combination inhibits histamine at the receptor of gastric parietal cells, reducing gastric acid secretion.

Adult dose 800mg po bid or 600mg po qid or 300mg po qid: not to exceed 1200mg/d (Netzer *et al*., 1999).

Antimicrobials

These drugs exert an antimicrobial effect on *H. pylori*.

(a) Amoxicillin (Amoxil, Trimox)

Semisynthetic penicillin antibiotic; interferes with synthesis of cell wall mucopeptides during active multiplication resulting in bacterial activity against susceptible bacteria. Adult dose 1000mg po bid for *H. pylori* eradication (Ford *et al*., 2004)

(b) Metronidazole (Flagyl)

Imidazole ring-based antibiotic active against anaerobic bacteria and some protozoa.

Adult dose 500mg po qid for *H. pylori* eradication (Vergara *et al*, 2005)

(c) Tetracycline (Sumycin)

Semisynthetic antibacterial agent derived from streptomyces culture. Effective against gram-positive and negative organisms as well as mycoplasmal, chlamydial and rickettsial infections.

Adult dose 500mg po divided bid/qid (Ford *et al*., 2004)

(d) Clarithromycin (Biaxin)

Macrolide antibiotics that inhibit bacterial growth, possibly by blocking dissociation of peptidyl - RNA from ribosomes.

Adult dose 500mg po qid for 14 days for *H. pylori* eradication (Ford *et al*., 2004)

(a) Bismuth subsalicylate (Pepto-Bismol)®.

Antacids

In recent times these are used in self-medications, they are not often used because larger doses are required to give desired effect. They include salt of aluminium, magnesium or calcium (Davidson, 1995).

Sucralfates

This is a basic aluminium salt of sucrose octasulphate. They act by protecting ulcer and stimulating renewal of epithelium to enhance defence mechanisms. It has very little effect on acid secretion (Davidson, 1995).

Prostaglandin Analogues

These enhance cytoprotection of the mucosal epithelial cells, low doses of misoprostol, an example, protects the mucosa against injury by noxious agents such as aspirin while high doses of the same drugs inhibit acid secretion and stimulates mucous and bicarbonate secretion (Davidson, 1995).

Antimuscarinic Agents

Muscarinic cholinergic antagonist can reduce basal gastric acid secretion by 40-50% stimulated secretion is inhibited to a lesser extent. Selective MI antagonists are usually used since their use is associated with fewer side effects like dry mouth, tachycardia when compared with use of non-selective muscarinic antagonist such as atropine. Example of these agents are pirenzepine and telenzipine. Both are hydrophilic and penetrate the blood barrier poorly and pirenzepine has produced comparable rate of healing duodenal ulcer in several chemical trials, and when used in maintenance dosages. It also appears to equal cimetidine for preventing the re-occurrence of peptic ulcer (Bruton, 1990).

2.5.5 Surgical Management

Endoscopy intervention is the primary mode of treating bleeding ulcers. Surgical management of peptic ulcer is generally reserved for refractory ulcers and bleeding ulcers that failed to respond to medical management (Hughes, 1993).

Endoscopic Therapy

Endoscopic therapeutic intervention is indicated for bleeding duodenal ulcers with high-risk signs e.g. active bleeding. Several tools are available to the endoscopist to achieve haemostasis; these tool include bipolar cautery, use of a heater probe or hemoclips, argon plasma coagulation, and local injection of epinephrine and other agents. Bipolar cautery and use of a heater probe both apply heat to the ulcer and cauterize the bleeding vessels. Hemoclip placement is a promising therapy but require skilled endoscopist and a lesion amenable to clip placement (Bardou *et al*, 2003). Injection of epinephrine achieves hemostasis through vasoconstrictive effect of epinephrine (Bardou *et al*., 2003).

Argon plasma coagulation uses heat to achieve hemostasis. One group compared argon plasma coagulation with heater probe and found no difference in incidence of rebleeding or the need for surgical intervention. In ulcer with high risk signs, endoscopic combination therapy with epinephrine injection and hemoclip placement is superior to injection alone (Lo, 2006; Cipolletal *et al*., 1998).

Urgent Surgical Management

The indication for urgent surgery includes the following:

i) Failure to achieve hemostasis endoscopically;

ii) Recurrent bleeding despite endoscopic attempt;

iii) Perforation

(Barkun *et al*., 2003)

Elective Surgical Management

The indications for elective surgical management include:

i) References to medical treatment;

ii) Intolerance to medications; and

iii) Non-compliance with medication

(Barkun *et al*, 2003)

Elective Surgical Approach

Vagotomy: Vagotomy involves resection of the vagus nerve which eliminates the autonomic stimulation of parietal cells. Historically, a truncal vagotomy was performed;

however, this led to gastric atony and subsequent stasis in as many as 20% of patient currently selective vagotomy are the procedure of choice. Selective vagotomy preserves the celiac and hepatic branches of the vagus nerve, thus decreasing the incidence of gastric atony. However, a gastric drainage procedure (.e.g. pyloroplasty) remain an essential component of this surgical approach. Highly selective vagotomy result in denervation of the parietal cells but preserves nerves supplying the pylo-antral region (Schaffattoky *et al*., 1999). The Billroth I and Billroth II are the two types of truncal vagotomy and antrectomy. These surgical approaches carry a mortality rate of approximately 1% and are currently performed much less frequently (Soll, 1996)..

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Animals

A total of eighty (80) adult male albino rats were used in this study. The animals were obtained from the animal house of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Their weight ranges from 165-180 grams at the time of the research. They were divided into 4 groups as follows;

Group I (Control Group)

(i) Five (5) rats received subcutaneous distilled water (2ml/rat) to study gastric ulceration.

(ii) Five (5) rats received subcutaneous distilled water (2ml/rat) to study basal gastric secretion.

Group II (Indomethacin-treated Group)

(i) Five (5) rats received indomethacin 20mg/kg S.C. for study of gastric ulcer formation

(ii) Five (5) rats received indomethacin 20mg/kg S.C. followed by pyloric ligation for the study of effect of indomethacin on gastric secretion.

Group III (Extract-treated Group)

A total of 30 rats were used and are subdivided as follows:

(i) Ten (10) rats received two different doses of extract (100 & 200mg/kg SC) to study their effects on gastric secretion (5 rats for each dose).

(ii) Ten (10) rats received two different doses of extract (100 & 200mg/kgSC) when given 30 minutes prior to indomethacin to study their effects on gastric secretion (5 rats for each dose).

(iii) Ten (10) rats received two different doses of extract 100 and 200mg/ Kg S.C. to study the effect on indomethacin-induced gastric ulceration (5 rats for each dose).

Group IV (Cimetidine-treated group)

A total of 30 rats were used, and are subdivided as follows:

(i) Ten (10) rats received two different doses of cimetidine (50 and 100mg kg SC) to study the effect on gastric secretion (5 rats for each dose).

(ii) Ten (10) rats received two different doses of cimetidine (50 and 100mg/kg SC) when given 30minutes prior to indomethacin, to study the effect on gastric secretion (5 rats for each dose).

(iii) Ten (10) rats received two different doses of cimetidine (50 and 100mg/kg SC) to study the effect on indomethacin-induced gastric ulceration (5 rats for each dose).Cimetidine was administered 30 minutes before indomethacin.

3.1.2 Drugs and Chemicals

Drugs

i) Indomethacin (Cheisi) ampoule used in a dose of 20mg/kg S.C. as reported by Okabe *et al*., (1977) and El-Sokkary *et al*., (1991a).

ii) Cimetidine (Lek Pharma.Slovenia) in a dose of 50mg/kg S.C.(Moron *et al*.,1982) and 100mg/kg S.C. (Satoh *et al*.,1983).

Chemicals

i) Sodium hydroxide (NaOH) (BDH Poole, England) was used for the preparation of 0.01N NaOH solution.

ii) Phenol red (BDH Poole, England) powder was used for the preparation of phenol red indicator.

iii) Chloroform (BDH Poole, England).

3.1.3 Plant Material

The *Hibiscus esculentus* (Linn) pods were bought from Samaru local market in Zaria. It was identified at the Department of Biological Sciences, Ahmadu Bello University, Zaria, by Mr. Galla, where a voucher specimen number (607) was deposited for future reference.

3.2 METHODS

3.2.1 Extraction of the Plant Material

The aqueous extraction procedures were carried out at the Department of Pharmacognosy laboratory, Ahmadu Bello University, Zaria

The room dried Okra pods were crushed into powder with pestles and mortar. Then, mixed with distilled water and put inside a soxhlet extractor steamed and the dried residue collected, weighed into various desired concentrations and kept ready to be used.

3.2.2 Identification of Chemical Constituents

Test for Reducing Sugars

1.0g of the aqueous extract of the Hibiscus esculentus was taken into a test tube. This was diluted with 2.0ml of distilled water followed by addition of Fehling's solution (A+B) and the mixture warmed. Brickred precipitate at the bottom of the test tube denotes the presence of reducing sugars (Brain and Turner, 1975).

Test for Resins

1.0g of the aqueous extract of the *Hibiscus esculentus* was dissolved in 1.0ml of acetic anhydride. One drop of concentrated sulphuric acid was added. Appearance of purple colour which rapidly changes to violet would indicate the presence of resins (Evans, 1994).

Test for Flavonoids

1.0g of the aqueous extract of the Hibiscus esculentus was dissolved in 50% methanol by heating. 5-6 drops of concentrated hydrochloric acid and magnesium were added. Appearance of a red colour would indicate the presence of flavonosides (Cuilei, 1994).

Test for Saponins

0.5g of the okra extract powder was taken into a test tube. 50ml of water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes and above would indicate the presence of saponins (Brain and Turner, 1975).

Test For Steroid Glycosides

1.0g of the aqueous extract of the okra diluted was taken into a test tube and evaporated to dryness. The residue was dissolved in acetic anhydride and chloroform was then added. By means of pipette, concentrated sulphuric acid was added by the side of the test tube. A brownish ring of the interface of the two liquids and the presence of violet colour in the supernatant layer would indicate the presence of steroids (Ciulei, 1994).

Test for Tannins

1.0g of the aqueous okra extract was diluted with distilled water in a test tube. 2-3 drops of 5% ferric solution was added. A green or blue-black combination indicates the presence of tannins (Ciulei, 1994).

Test for Alkaloids

1.0g of the aqueous extract of the okra powder in two separate test tubes, 2-3 drops of Dragendoff's and Mayer's reagents were separately added. An orange red precipitate/turbidity with Dragendoff's or white precipitate with Mayer reagents would denote the presence of alkaloids (Ciulei, 1994).

3.2.3 Acute Toxicity Testing

LD_{50} determination was conducted – using the method of Lorke (1983). In the initial phase, male rats were divided into three groups of 3 rats each. They were treated with the aqueous extracts of *Hibiscus esculentus* at doses of 10, 100 and 1000mg/kg subcutaneously. Animals were observed for 24 hours and the number of death(s) was recorded. In the second phase, they were grouped into three groups of one rat each and treated with the aqueous extract at doses of 1600,2900,5000 subcutaneously. Animals were observed for four hours and the final LD_{50} was calculated.

3.2.4 Induction of Gastric Ulceration

After the 48 hours starvation, the animals were weighed and maintained in their individual cages. Then indomethacin 20mg/kg subcutaneous was injected and the animals were deprived from both food and water for 7 hours (Urushidani *et al.,* 1979). The animals were later on sacrificed by decapitation (Satoh *et al.,* 1983). Their stomachs opened along the greater curvature, rinsed slowly with water, then stretched out as much as possible on a Whatman's filter paper. The ulcerated areas on each stomach measured with a transparent (mm) ruler scale, and the results for each group expressed in mm of mean ulcer ± SE (ulcer index) (Scepovic and Radmanovic, 1984).

The percentage preventive index was later on calculated according to the method of Hano *et al*. (1976), which is expressed as:

$$\text{Preventive Index (\%)} = \frac{\text{(U.I Indomethacin} - \text{U.I Extract/drug plus) indomethacin}}{\text{U.I. indomethacin}} \times 100$$

Quantitative Assessment of Mucosal Damage

The stomachs were opened along the greater curvature, rinsed slowly with running water. They were then stretched out as much as possible, by the use of pins and filter paper on a ceiling board.

The ulcerated areas in each stomach were measured with a transparent (mm) ruler scale, then, result of each group was expressed as ulcer index (UI) in mm of mean ulcer ± SE standard error (Scepovic and Radmanovic, 1984).

The degree of ulceration was expressed as ulcer index (U.I). Ulcer index was calculated by multiplying ulcer score by 100 (Robert *et al.*, 1968). Ulcer score for each group was calculated by dividing the total number of ulcers in each group by the total number of rats in that group (Robert *et al.*, 1968).

3.2.5 Collection of Gastric Secretion

The gastric juice was collected according to the technique of Shay *et al*. (1954) as modified by Levine (1965) where oesophageal ligation was avoided. The animals were fasted for 48 hrs to ensure complete emptying of the stomach, but allowed water ad libitum. Each of the animals was weighed at the end of the fasting period.

Light anaesthesia was used, their abdomen opened via a midline incision and the stomach identified. A pyloric ligature was made using the thread with care to avoid damage to the blood vessels or traction to the stomach. The abdomen closed by suture, cleaned thoroughly with distilled water or saline. The anaesthesia discontinued and the animals allowed to recover.

After 3 hours, the animals were sacrificed, abdomen of each of the animals was opened. Then the oesophagus was ligated, and the stomach was removed and washed with distilled water or saline. An opening along the greater curvature was made, and the gastric content drained into a graduated centrifuge tube, the volume noted, then centrifuged at 3000rpm for 15 minutes.

Precautions during the Pyloric Ligation and Collection of the Gastric Secretion

i. All rats should be of the same strain, healthy and well-fed with a uniform standard diet (Shay *et al.*, 1954).

ii. Anaesthesia should be used at operation only and not throughout the test period as described by Raymond et al., (1995).

iii. Delicate and gentle handling of the blood vessels must be taken into consideration (Shay *et al*., 1954).

iv. After the operation, rats were kept in separate cages and received nothing during the remaining time of the experiment.

v. The preoperative starvation period of 48 hrs was chosen, by Shay et al (1954) because starvation for 24 hrs was inadequate to ensure complete gastric emptying of the rats, and 72 hours according to Madden et al. 1951, could affect the rate of gastric secretion and lower the gastric mucosal resistance, with consequent haemorrhagic erosions and buffering of gastric juice.

3.2.6 Analysis of Gastric Juice

Determination of each sample volume after centrifugation

The volume of 3 hours gastric secretion was calculated after being exposed to centrifugation for sufficient time (10-15min).

Determination of titratable acidity and total mean acid output

A given volume of the gastric juice (0-2ml) was titrated against 0.01N NaOH. An end point of pH 7.0 as determined colorimetrically by Phenol red was used (Grossman, 1973; Davenport, 1977).

The value was calculated as micro-equivalents per litre (Meq/L) which is equal to the number of millitres (ml) of 0.01 N NaOH required to neutralize 1ml of gastric juice.

$$\text{Titritable Acidity} = \frac{\text{Volume of 0.01 N NaOH (ml) which neutralizes 1ml of gastric juice}}{10}$$

Total titritable acid output was calculated as unit-equivalent per 3 hours by multiplying the volume of gastric juice in ml by titritable acidity in (M.eq/L) divided by 1000 (Okabe *et al.,* 1975).

3.2.7 Statistical Analysis

All data was expressed as mean ± S.E.M. The data collected was analyzed using Analysis of Variance (ANOVA) followed by post hoc analysis by Duncan and Scheffe.

CHAPTER FOUR

RESULTS

4.1 PHYTOCHEMICAL SCREENING

Preliminary phytochemical analysis of the extract revealed the presence of flavonoids, glycosides, and phenolics.

4.2 ACUTE TOXICITY STUDIES

The oral and subcutaneous LD_{50} study of the extract in mice was found to be above 5000 mg/kg.

4.3 GASTRIC MUCOSAL INTEGRITY STUDIES

The results of the gastric mucosal studies are presented below:

4.3.1 Control Group

The results of this group revealed that the mucosa was found to be normal and the ulcer index (UI) was recorded as zero in table (1)

4.3.2 Indomethacin-treated Group

Indomethacin (20 mg/kg S.C.) produced severe gastric mucosal ulceration restricted to the corpus with an ulcer index of 16.6 ± 4.4. The ulcers appeared in the form of streaks and/or groups and went deep in the mucosa with severe necrotic and hemorrhagic bases as represented in table (1).

Table 1: Ulcer index (mm) and preventive index (%) for control and I indomethacin treated rats

Parameters Groups	Ulcer Index (mm)	Preventive Index (%)
Control	0.00 ± 0.0 (5)	100 (5)
Indomethacin 20 mg/kg	16.6 ± 0.1 (5) P<0.001 ***	0 (5) P<0.001 ***

- P<0.05- Significant;
- ** = P<0.01- Highly Significant;
- ***= P<0.001 - Very Highly Significant

4.3.3 Cimetidine Plus Indomethacin-Treated Group

The data represented in table (3) was obtained following the administration of cimetidine in the doses of 50 and 100 mg/kg followed by Indomethacin for their effect on gastric mucosa. The ulcers were negligible on the peripheral parts of the corpus, but the gastric mucosa in between the ulcers was normal.

4.3.4 *Hibiscus esculentus* Extract Plus Indomethacin-Treated Group

Hibiscus esculentus extract administered in the doses of 100 and 200 mg/kg produced the following results presented in table (3):

4.4 GASTRIC SECRETION STUDIES

The gastric juice collected three (3) hours after pyloric ligation in different animal groups was subjected to the studies of volume, titritable acidity and total acid output. The results obtained have been summarized in the following different tables.

4.4.1 Indomethacin-Treated Group

The different parameters of gastric secretion were studied and the results obtained are presented in table 5 It is noticed that indomethacin significantly affects the acid output, but titritable acidity is not affected significantly.

4.4.2 Histopathological Studies

Table 2: Ulcer index (mm) and preventive index (%) for control, indomethacin and cimetidine treated rats

Parameters Groups	Ulcer Index (mm)	Preventive Index (%)
Control	0.00 ± 0.0 (5)	100 (5)
Indomethacin 20 mg/kg	16.6 ± 0.1 (5) P<0.001 ***	- (5) P<0.001 ***
Cimetidine 100 mg/kg plus Indomethacin	2.10 ± 1.28 (5) P<0.05 *	87 (5) P<0.05 *
Cimetidine 50 mg/kg	2.60 ± 1.37 (5) P<0.05 *	78 (5) P<0.05 *

Table 3: Summary of the different parameters in gastric ulcers study

Parameters Groups	Ulcer Index (mm)	Preventive Index (%)
Control	0.00 ± 0.0 (5)	100 (5)
Indomethacin 20 mg/kg	16.6 ± 0.1 (5) P<0.05 ***	- (5) P<0.05 ***
Okra 200 mg/kg plus Indomethacin	0.10 ± 0.1 (5) P>0.05	99 (5) P>0.05
Okra 100 mg/kg plus Indomethacin	4.10 ± 2.4 (5) P<0.05 **	75 (5) P<0.05 **

Table 4: Summary of the different parameters studied for 3 hours gastric secretion for control and Indomethacin-treated groups

Parameters / Groups	Volume ml/ 3 hours	Titritable acidity mEq/L	Acid output μEq/h
Control	3.70 ± 0.5 (5)	30.00 ± 5.2 (5)	35.20 ± 5.0 (5)
Indomethacin 20 mg/kg	1.04 ± 0.4 (5) P<0.05 *	23.40 ± 4.6 (5) P<0.05 *	8.60 ± 2.0 (5) P<0.05 *

Table 5: Summary of the different parameters studied for 3 hours gastric secretion for control, Indomethacin and cimetidine treated groups

Parameters / Groups	Volume ml/ 3 hours	Titritable acidity mEq/L	Acid output μEq/h
Control	3.70 ± 0.5 (5)	30.00 ± 5.2 (5)	35.20 ± 5.0 (5)
Indomethacin 20 mg/kg	1.04 ± 0.4 (5) P<0.05 *	23.40 ± 4.6 (5) P<0.05 *	8.60 ± 2.0 (5) P<0.05 *
Cimetidine 100 mg/kg	1.02 ± 0.1 (5) P<0.05 *	23.60 ± 4.7 (5) P<0.05 *	8.92 ± 1.4 (5) P<0.05 *
Cimetidine 50 mg/kg	1.04 ± 0.1 (5) P<0.05 *	23.00 ± 5.3 (5) P<0.05 *	7.24 ± 1.3 (5) P<0.05 *
Cimetidine 100 mg/kg plus Indomethacin	2.14 ± 0.3 (5) P<0.05 *	33.40 ± 1.9 (5) P<0.05 *	24.50 ± 4.7 (5) P<0.05 *
Cimetidine 50 mg/kg plus Indomethacin	2.12 ± 0.2 (5) P<0.05 *	41.40 ± 4.3 (5) P<0.05 *	30.14 ± 5.4 (5) P<0.05 *

Table 6: Summary of the different parameters studied for 3 hours gastric secretion for control, Indomethacin, extract and cimetidine-treated groups

Parameters / Groups	Volume ml/ 3 hours	Titritable acidity mEq/L	Acid output µEq/h
Control	3.70 ± 0.5 (5)	30.00 ± 5.2 (5)	35.20 ± 5.0 (5)
Indomethacin 20 mg/kg	1.04 ± 0.4 (5) P<0.05 *	23.40 ± 4.6 (5) P<0.05 *	8.60 ± 2.0 (5) P<0.05 *
Extract 200 mg/kg	1.32 ± 0.1 (5) P<0.05 *	28.00 ± 1.5 (5) P<0.05 *	12.08 ± 1.2 (5) P<0.05 *
Extract 100 mg/kg	1.12 ± 0.2 (5) P<0.05 *	20.00 ± 1.5 (5) P<0.05 *	7.00 ± 0.1 (5) P<0.05 *
Extract 100 mg/kg plus Indomethacin	1.22 ± 0.2 (5) P<0.05 *	15.20 ± 3.2 (5) P<0.05 *	5.90 ± 1.2 (5) P<0.05 *
Extract 200 mg/kg plus Indomethacin	1.44 ± 0.2 (5) P<0.05 *	20.00 ± 3.0 (5) P<0.05 *	9.52 ± 1.5 (5) P<0.05 *
Cimetidine 100 mg/kg plus Indomethacin	2.14 ± 0.3 (5) P<0.05 *	33.40 ± 1.9 (5) P<0.05 *	24.50 ± 4.7 (5) P<0.05 *
Cimetidine 50 mg/kg plus Indomethacin	2.12 ± 0.2 (5) P<0.05 *	41.40 ± 4.3 (5) P<0.05 *	30.14 ± 5.4 (5) P<0.05 *

HISTOPATHOLOGICAL REPORT CONTROL GROUP (2ML Distilled Water)

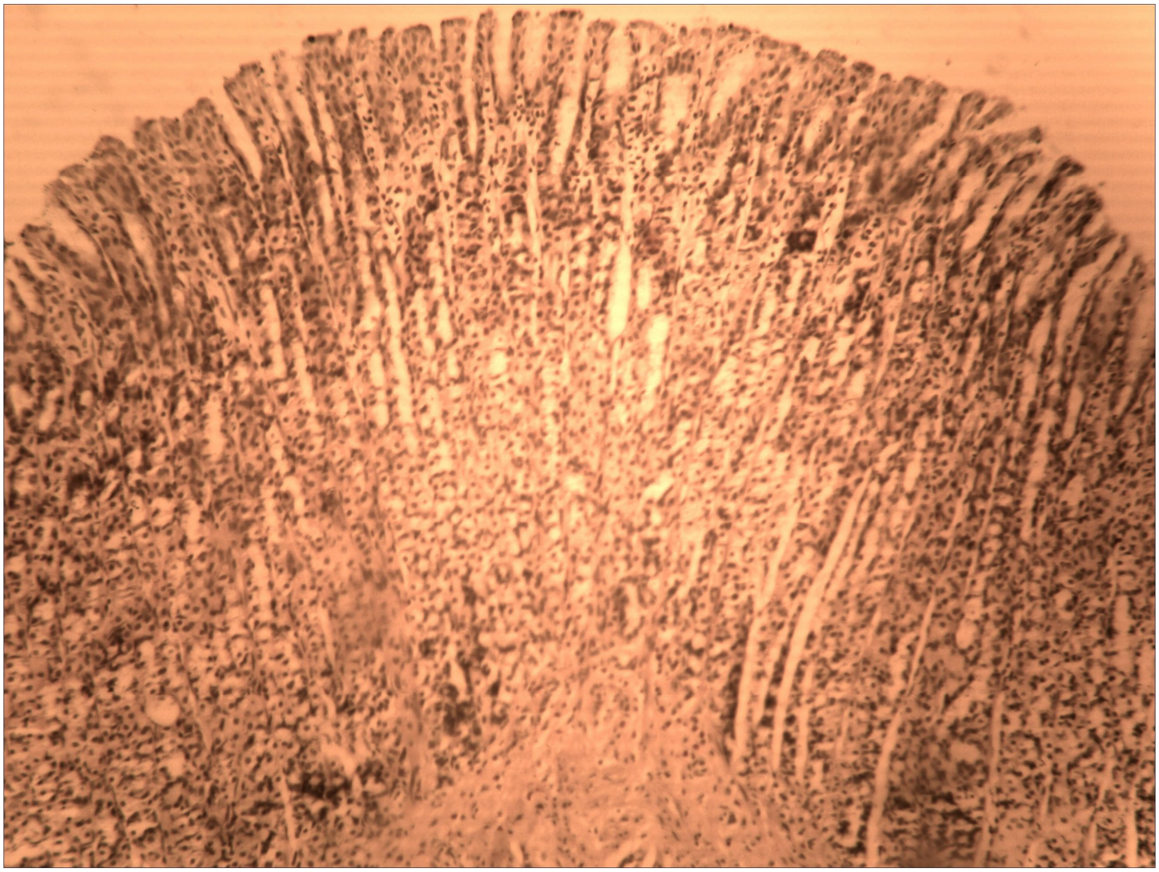


Plate 4.1: Photomicrograph of a section of stomach from a control rat. It reveals normal histological architecture of gastric mucosa with no observable microscopic lesion (Mag. x 100)

***HIBISCUS ESCULENTUS* EXTRACT (200mg/kg) PLUS INDOMETHACIN 20mg/kg**

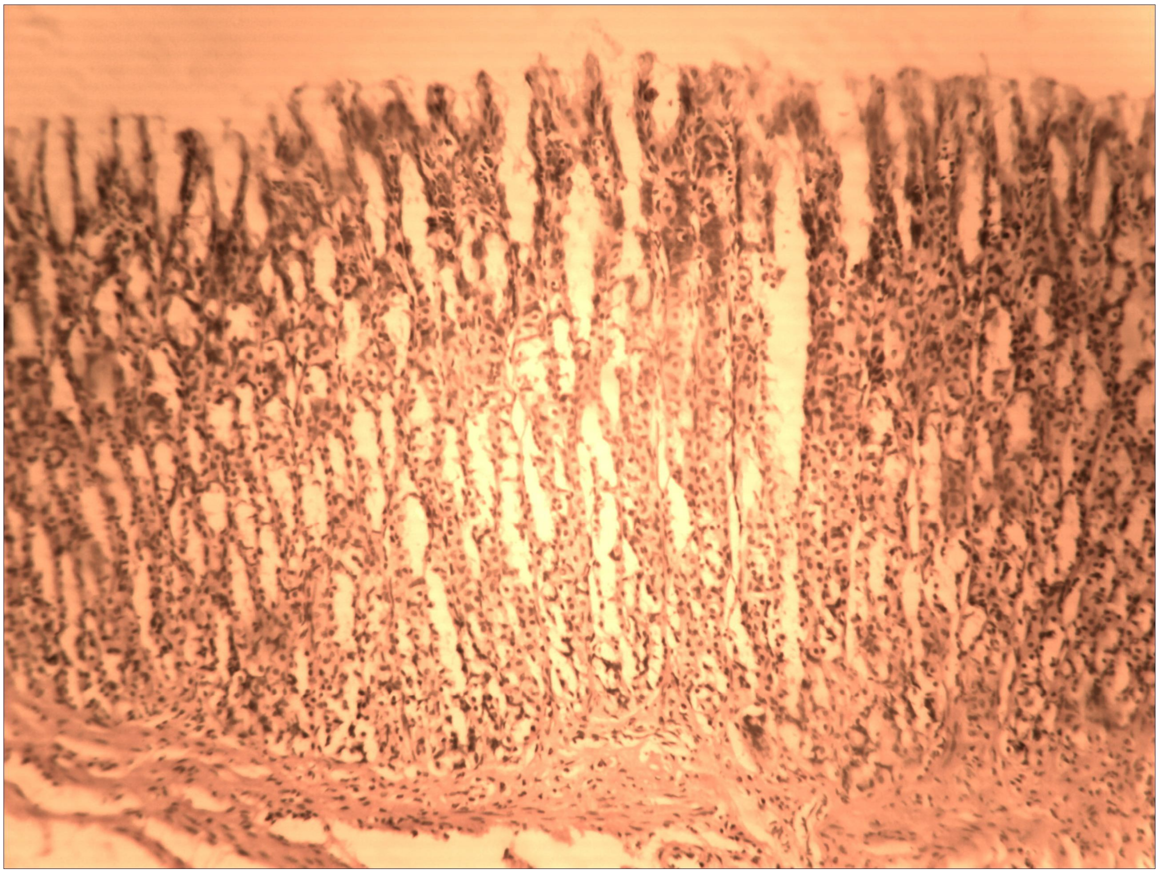


Plate 4.2: Photomicrograph of a section of stomach from a rat treated with Okra extract 200mg/kg plus indomethacin 20mg/kg showing ulceration of superficial gastric epithelium and enlargement of gastric pits evident (Mag. x 100)

***HIBISCUS ESCULENTUS* EXTRACT (100mg/kg) PLUS INDOMETHACIN 20mg/kg**

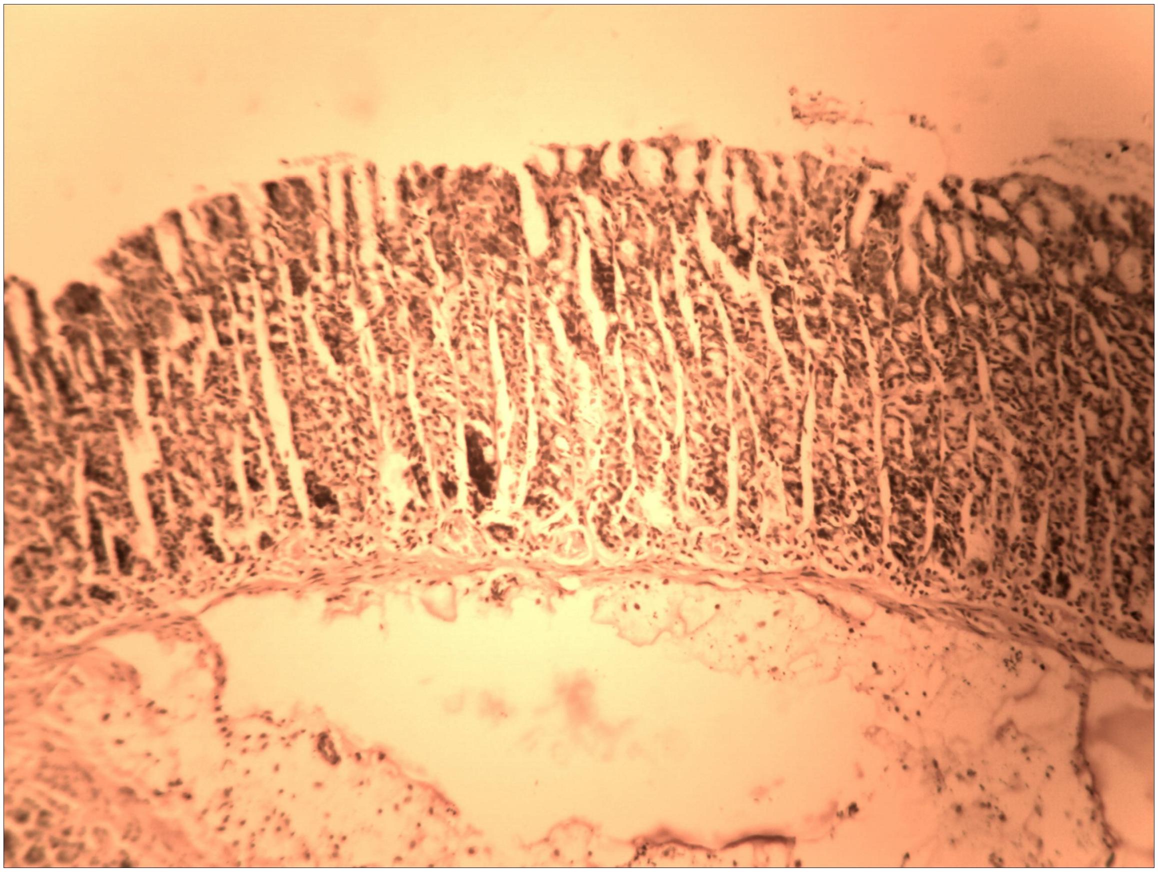


Plate 4.3: Photomicrograph of a section of stomach from a rat treated with Okra extract 100mg/kg plus indomethacin 20mg/kg showing severe ulceration and necrosis of superficial gastric epithelium and deep ulceration extending through the mucosa (Mag. X 100)

CIMETIDINE (100mg/kg) PLUS INDOMETHACIN (200mg/kg)

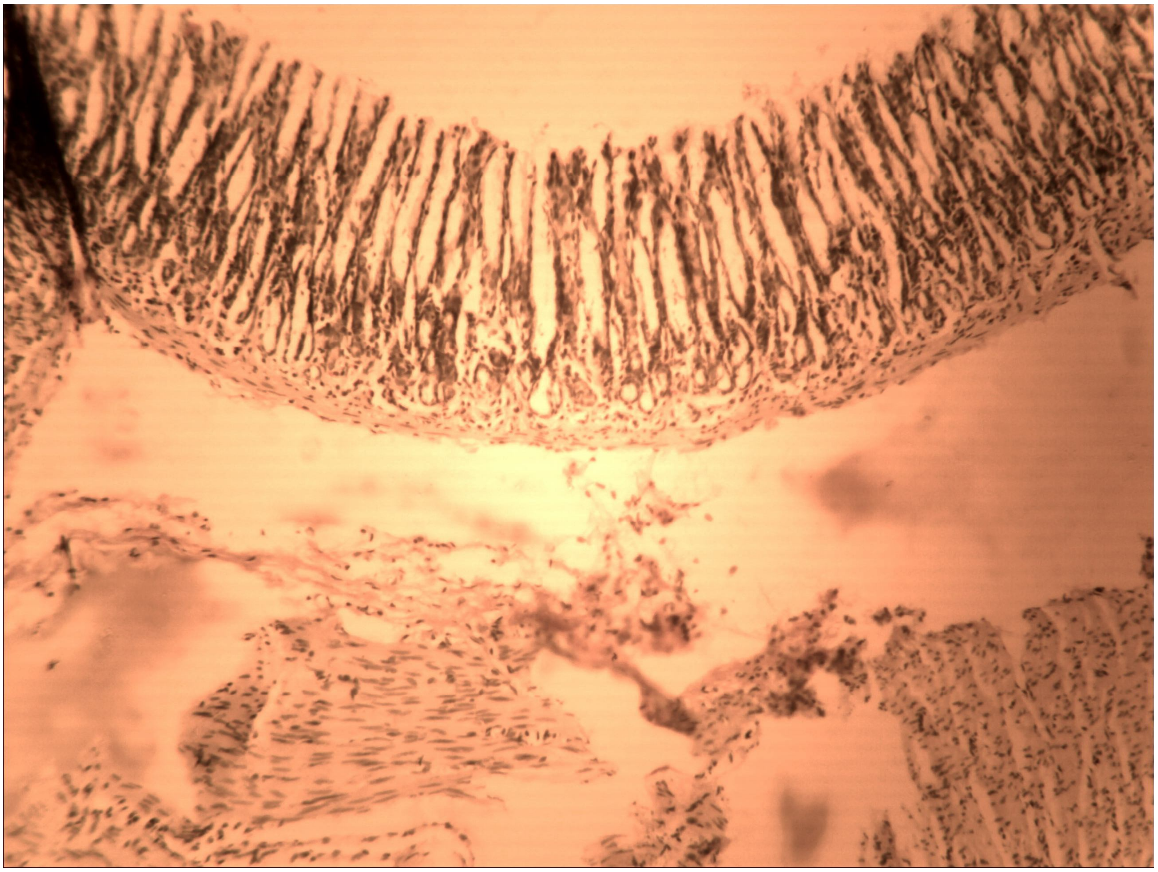


Plate 4.4: **Photomicrograph of a section of stomach from a rat treated with cimetidine 100mg/kg plus indomethacin 20mg/kg showing severe necrosis and ulceration of superficial gastric epithelium; enlargement of gastric pits plus deep ulcerations stretching through the mucosa (Mag. X 100)**

INDOMETHACIN (20mg/kg)

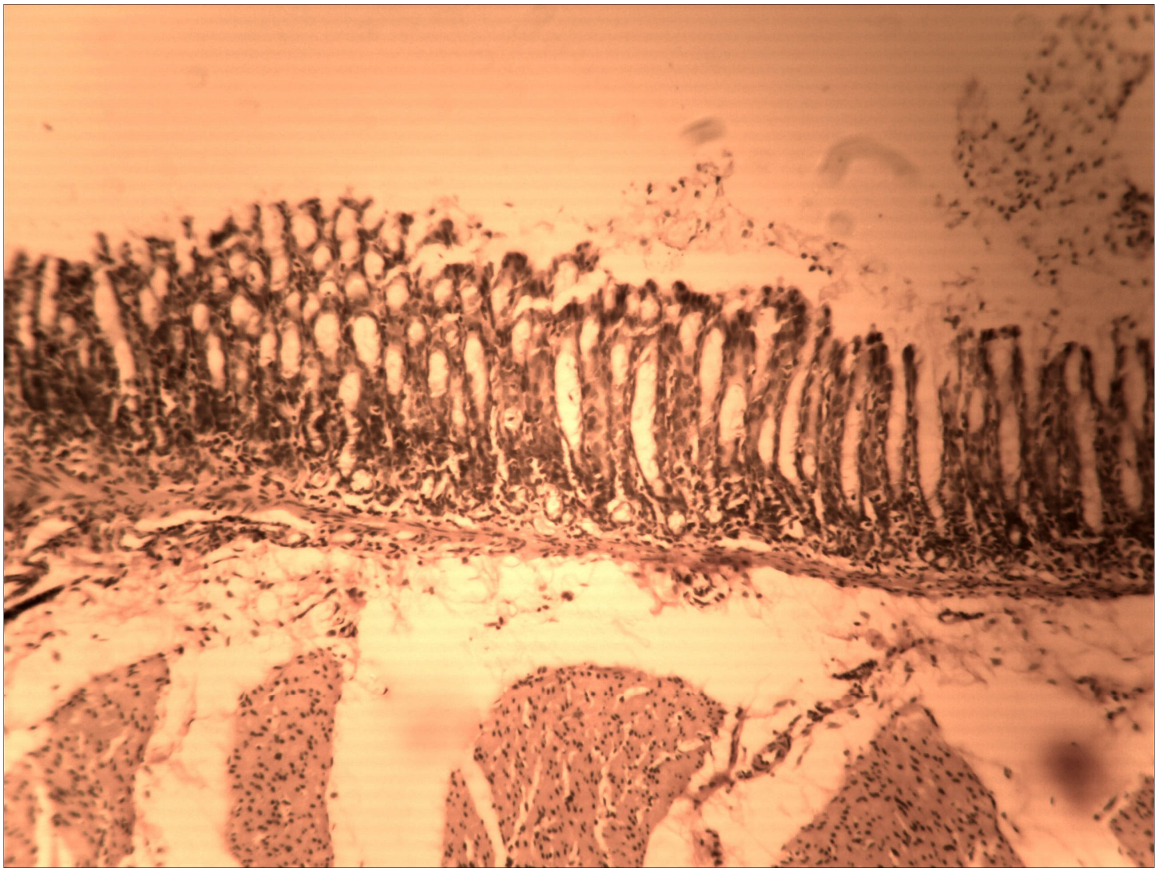


Plate 4.5: Photomicrograph of a section of stomach from a rat treated with indomethacin 20mg/kg showing severe ulceration and necrosis of superficial gastric epithelium plus enlargement of gastric pits and deep ulcerations stretching through the mucosa (Mag x 100)

CHAPTER FIVE

DISCUSSION

The models of gastric ulcer induction according to the methods of Shay et al., (1945) and Raymond *et al.* (1995) were used in this study, hence the use of indomethacin as the ulcer induction agent. The results indicated the aqueous *Hibiscus esculentus* extract to be an important source of antiulcer agents. The ulceroprotective and gastric secretion effects of *Hibiscus esculentus* based on the results obtained in this study can be physiologically suggested as such.

5.1 EFFECTS OF INDOMETHACIN ON GASTRIC MUCOSA AND GASTRIC SECRETION PARAMETERS

Non steroidal anti-inflammatory drugs and aspirin (collectively called NSAIDs) use, is the second most common etiologic factor for peptic ulcer disease and a major factor for peptic ulcer complications (Voutilainen *et al.*, 2001).Indomethacin which is one of the NSAIDs have the ability to cause gastroduodenal ulceration, and this effect is related to the ability of these agents to suppress prostaglandin synthesis (Wallace, 2000).

Experimental evidence has shown that incidence of ulcers in patients that take this drug is as a result of failure of the mucosa to develop resistance to the damaging effect of these drugs (Wallace *et al.*, 2005). Reports have indicated that gastroduodenal protection by prostaglandins is due to increasing mucosal resistance, as well as the decrease in aggressive factors, mainly acid and pepsin (Aly, 1987). Prostaglandins normally protect the gastrointestinal mucosa from damage by maintaining mucosal blood flow and increasing mucosal secretion of mucus and bicarbonate. Blockade of COX-1 activity by NSAIDs reduces prostaglandin synthesis and thus lowers gastrointestinal mucosal blood flow and secretion of mucus and bicarbonate (Feldman, 2002).Therefore, probable cytoprotective mechanism can be attributed to increased mucus and prostaglandin synthesis. Gastric mucosal lesions in the form haemorrhagic red streaks, erosions and/or ulcers were observed following subcutaneous administration of indomethacin as seen in the result of this work. Unlike *Helicobacter pylori*- related peptic ulcers, which more often occur in the duodenal bulb, NSAIDs -induced ulcers typically occur in the stomach. Though, NSAIDs can also cause ulcers in the jejunum, ileum, or colon, areas where there is little or no acid pepsin (Feldman, 2002). This work further delineated the action of indomethacin, in that the gastric lesions were confined to the corpus of the gastric mucosa along the longitudinal axis of the stomach, with the antrum and duodenum unaffected. Indomethacin-induced lesions were confined to the corpus mucosa of the rat stomach (Takeuchi *et al.*, 1989; El-Sokkary *et al.*,1991a; Filaretova *et al.*, 2002; Okasha *et al.*, 2008).This is consistent with our results. However, Stables *et al*., (1993) reported discernible, discrete brown/black lesions on the mucosal surface, primarily in the antral region of the rat’s stomach following indomethacin administration, which is contrary to our studies.

Several studies reported the necrotizing effect of indomethacin and other NSAIDs on the gastric mucosa as reported in this work. Soll *et al.*, (1989) and also El-Sokkary *et al.*, (1991a) reported haemorrhage as the most common complication of NSAIDs ,as such rats that received only indomethacin showed haemorrhagic gastric secretion.

Gastric secretion as affected by indomethacin in this study is insignificant ($P<0.05$). Important gastric secretion parameters analyzed including the volume and titritable acidity showed a decrease which is insignificant ($P<0.05$). But, there is a marked decrease in acid output. These results are consistent with the reports of Nicoloff (1967), Kobayashi *et al.*, (1985) and El-Sokkary and his colleagues (1991a) who reported significant decrease in canine gastric acid output as induced by indomethacin. However, indomethacin within the therapeutic dose augments basal acid output (Levine and Schwartzel, 1984; Khayyala *et al.*, 2006), which is contradictory to our results. Though, other studies showed that at ulcerogenic dose, indomethacin had no effect on basal or stimulated acid output (Ueki *et al.*, 1988; Filaretova *et al.*, 2002). Hence, from previous studies and ours, the role of indomethacin on gastric secretion is yet to be settled.

Acid output which is one of the principal aggressive factors has been decreased ($P<0.05$) by indomethacin in this study, in consistent with studies of El-Sokkary *et al.* (1991a). Therefore, the role of acid in ulcerogenesis caused by indomethacin need to be further evaluated.

Synthetic NSAIDs like aspirin, indomethacin cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H^+ ions, resulting in over production of leukotrienes and other products of 5-lipoxygenase pathway (Rao *et al.,* 2000).

Mersereau *et al.* (1982) reported the role of glycopyruvic response in the mechanism of hypermotility induced by NSAIDs. Indomethacin at an ulcerogenic dose causes gastric hypermotility which is a key response in the aetiopathogenesis of gastric mucosal damage (Takeuchi *et al.*,1986; El-Sokkary *et al.*,1993).

5.2 EFFECTS OF CIMETIDINE AS STANDARD ANTI-ULCER DRUG ON UCOSAL DAMAGE AND GASTRIC SECRETION PARAMETERS

Cimetidine, a H_2-receptor blocker that is known to possess ulceroprotective properties in the gastric mucosa, has been evaluated in this study at different doses of 50 and 100mg/kg.This is to determine whether protection of the gastric mucosa could be provided against acute dose of indomethacin at 20mg/kg.It was found out that acute gastric mucosal damage induced by indomethacin was significantly reduced ($P<0.05$) by cimetidine at the above doses. In the ulcer indices, cimetidine at 100mg/kg produces a smaller ulcer index, compared to the 50mg/kg.

Consequently, the preventive index of the higher dose (100mg/kg) was higher than that of the smaller dose (50mg/kg).Therefore, prevention of gastric damage caused by indomethacin by the use of cimetidine in this study was in dose –dependent manner. These is in agreement with the studies of Okabe *et al.*,(1977) where they reported marked inhibition of gastric damage induced by the adverse effects of aspirin and indomethacin due to cimetidine. Similarly, El-Sokkary *et al.* (1991b) and Mequanente et al. (2006) reported on the gastroprotective effect of cimetidine at 100mg/kg in rats. However, there are documented reports of cimetidine been effective against indomethacin -induced ulcers at higher doses above 100mg/kg (Garginella *et al.*, 1985; Bauer *et al.*, 1986).

Studies on gastric juice parameters, revealed a decrease in a dose-dependent manner in all the parameters in this current work. We found out that volume of gastric juice, titritable acidity, acid output were all significantly reduced ($P<0.05$) by cimetidine. This results are in agreement with the studies of Okabe *et al.* (1977); Satoh *et al.* (1984);Roth *et al.* (1987); Ueki *et al.*(1988) and El-Sokkary *et al.* (1991b) who reported that at a dose of 100mg/kg, cimetidine decreased both volume of gastric juice and acid output. Thompson and Mahakai (1985), in a study that is consistent with the present study, reported a reduction in the output of basal and stimulated acid and pepsin secretion in healthy volunteers and patients with gastric and duodenal ulcers induced by cimetidine.

Protection against indomethacin-induced relapse of mini-ulcers has been reported to be produced by cimetidine as documented by Satoh *et al*.(1984) using rats model of gastric ulcer relapse. This gastroprotective effect of cimetidine leading to mucosal healing has been shown to be associated with increasing PGE_2 synthesis in mini-ulcers post indomethacin therapy. Evidences suggested that PGE_2 is angiogenic in- vivo and controls gastric epithelial cell proliferation (Form and Auerbach, 1983). Cimetidine also promotes synthesis of inducible form of cyclooxygenase (Kawaguchi *et al*., 1995). Therefore, it can be proposed that cytoprotection leading to decreased susceptibility to recurrent mucosal breakdown is facilitated by angiogenesis and increased prostaglandin synthesis. But, the exact role of cimetidine in this process is still cloudy.

5.3 EFFECTS OF AQUEOUS EXTRACT OF *Hibiscus esculentus* ON MUCOSAL DAMAGE AND GASTRIC SECRETION PARAMETERS

In the current study, the aqueous extract of *Hibiscus esculentus* inhibited the production of indomethacin-induced ulcers and also have significant ($P<0.05$) effects on gastric acid secretion induced by indomethacin. The different doses of the aqueous extract of *Hibiscus esculentus* of 100 and 200mg/kg were administered 30 minutes prior to indomethacin.

Studies on gastric mucosal ulcerations revealed that the 2 different doses of 100 and 200mg/kg of the extract have shown a reduction in the ulcer index when compared to the control and indomethacin treated groups. It was more significant ($P>0.05$) at 200mg/kg. This reduction was dose – dependent with higher ulcer index at the lower dose of 100mg/kg. Consequently, the preventive index was much higher in the larger dose of 200mg/kg, compared to the 100mg/kg dose. So, the prevention also follows a dose dependent fashion, with the highest protection at the 200mg/kg.Interestingly,the reduction in the ulcer index with a subsequent significant gastroprotection afforded by the 200mg/kg of the aqueous extract of *Hibiscus esculentus* is significantly higher ($P<0.05$) than in the cimetidine most effective dose (100mg/kg) as reported by this study.

On the gastric secretion parameters, the two doses of 100 and 200mg/kg *Hibiscus esculentus* aqueous extract in our study revealed significant reduction ($P<0.05$) in all the parameters of gastric secretion tested when compared with the control group and also the indomethacin treated group. The reduction in the gastric secretion parameters in this study occurred in a dose-dependent manner with more reduction in the higher dose of 200mg/kg.

Hence, the aqueous extract of *Hibiscus esculentus* administered at the two different doses of 100 and 200mg/kg subcutaneously produced appreciable and significant effects on the different parameters of gastric mucosal damage and gastric secretion respectively. The effects exhibited by these two doses of the extract were shown to be dose dependent with the highest protection in the group that received 200mg/kg subcutaneously.

Proposed Mechanism of Action of the Different Phytochemicals found in the *Hibiscus esculentus* Aqueous Extract on Gastric Ulceration and Secretion

These studies indicated that *Hibiscus esculentus* may play a role in facilitating and enhancing gastric mucosal defensive factors. Anti-secretory potency was also demonstrated by *Hibiscus esculentus* using the pyloric ligature technique of Shay *et al*., (1954) at doses of 100 and 200mg/kg.These effect could suggest an anti-histaminergic potency. The preliminary phytochemical analysis of the Hibiscus esculentus extract used in this study revealed the presence of flavonoids, tannins and alkaloids. In these regard, there are several reports where flavonoids have shown cytoprotective and anti-secretory properties in different experimental models of gastric ulcer (Takase *et al.*, 1994; Martin *et al.*, 1998). These findings have contributed in explaining the anti-ulcer activity observed for the extract of *Hibiscus esculentus*. Therefore, the results obtained demonstrated that *Hibiscus esculentus* displays a good anti-ulcer activity, further supporting the gastroprotective effects of the flavonoids contained in the *Hibiscus esculentus* extract, hence contributing to its pharmacological validation.

The flavonoids have aroused considerable interest recently, because of their potential beneficial effects on human health as such they have been reported to possess antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumour and antioxidant activities (Burler and Miranda, 2003). These are polyphenolic compounds that are ubiquitous in nature. Different categories of flavonoids have been categorized according to some variations on their parent chemical structure. They are consumed daily in large amounts in a large variety of foods, principally in fruits, vegetables and beverages. They are present mainly in red wines (Arts *et al*., 2000; Landrault *et al.*, 2001), teas (Arts *et al*., 2000; Aherne and O-Brien., 2002), cacao products (Arts *et al.*, 2000; Hammerstone *et al*., 2000), and also in a lot of fruits such as apples, berries and grapes, which are particularly rich in procyanidins and flavonols (Fuleki and Ricardo da Silva, 1997). There is a considerable correlation between the antioxidant and the antiulcer activity of flavonoids (Galati *et al*., 2003).

Several researches have shown, support and confirmed the efficacy of several plant extracts for the treatment of gastroduodenal disease, and the anti-ulcerogenic effect of some of these plants is related to their flavonoid content, micronutrients activity, organic and inorganic compounds (Czinner *et al.,* 2001). Yesilada *et al.* (2000), further corroborated this study by highlighting that the antiulcerogenic activity of many plant species is related to their content of flavonoids. *Sparceum junceum* L., a species used in

Turkish folk medicine to treat gastric ulcer, contains glycosylated flavonoids with antioxidant activity. A flavonoid hypolaetin-8-glucoside present in the genus of several species of Sideritis also has significant anti-ulcerogenic activity (Villar *et al*., 1984). Another flavonoid present in *Musa sapirntum* L. protects the gastric mucosa from aspirin-induced lesions (Lewis *et al*., 1999). Sophoradin, a flavonoid derivative from *Sophora subprostata* has been reported to have good gastroprotective and ulceroprotective properties. The mechanism of action was thought to be possibly via an action on the production and metabolism of prostaglandins and mucus in the gastric mucosa (Konturek *et al.*, 1986).

Flavonoids have membrane stabilizing properties and also affect some processes in the intermediary metabolism and inhibit lipid peroxidation indifferent systems (Repetto and Liesuy, 2002). Some of them have shown to increase the mucosal content of prostaglandins and mucus in gastric mucosa with cytoprotective effects. Several of them prevent gastric mucosal lesions produced by various experimental ulcer models and protect the gastric mucosa from against different necrotic agents (Alanko, 1999).

Plants containing flavonoids are effective in preventing ulcerogenic lesions because of their antioxidant properties (Cody *et al*., 1986; Harborne andWilliams, 2000).
Several in vitro studies have indicated that flavonoids can scavenge dihydroxyl, peroxide superoxide radicals thereby affecting various steps in the arachidonate cascade via cyclooxygenase-$_2$ or lipooxygenase (Alanko, 1999). They were also identified to confer gastroprotection via inhibition of Ca^+ ions influx that signals the last step in the cell death

cascade, quenching of reactive oxygen species, and alteration in GSH metabolism (Ishige *et al*., 2001). Strong evidences emerged recently, that oxidation processes are involved in the mechanism of different gastric disorders including ulcerogenesis and this has further aroused interest in the antioxidant activity of flavonoids (Cook and Samman, 1996; La Casa *et al.,* 2000).They were further supported by many in vitro studies that characterized flavonoids as powerful antioxidants capable of efficient scavenging of both reactive oxygen and nitrogen species (Rice-Evans *et al.,*1996; Bagchi *et al*., 2003; Burda and Oleszek, 2001). Numerous investigations have concluded that flavonoids play a central role in the antiulcer and anti inflammatory activities of plants (Cook and Samman, 1996; Di Carlo *et al.,* 1999; Harborne and Williams, 2000).

Tannins were also screened to be present in *Hibiscus esculentus* and are reported to also possess gastroprotective and antiulcer effects. Tannins isolated from *S. cumini* have significant gastroprotective property (Ramirez, 2003). It has been shown that all of the effective gastroprotective plant extracts contained tannins and/or flavonoid (Glavin and Mikhail, 1976).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The work presented in this research work gives an insight into the role of okra on gastric mucosal protection and gastric secretion. The conclusions drawn are based on the results obtained from the study. From the results obtained, it is clear that the various factors involved in the maintenance of the gastric mucosal integrity are due to the phytochemicals obtained from the aqueous extract of *Hibiscus esculentus* that produced gastroprotective activity, therefore *Hibiscus esculentus* may be recommended.

RECOMMENDATIONS

We can therefore conclude by recommending that there should be further and in-depth study on the possible mechanisms of action of the aqueous extract of *Hibiscus esculentus*, and it's various fractions.

REFERENCES

Aherne, S.A. and O.Brien, N.M. (2002): Dietary Flavonols: Chemistry, Food content and Metabolism. *Nutrition*, 18:75-81.

Ahmed, P.N. and Rao, M.A. (1975): Induction of gastric ulcers in the forestomachectomized albino rats by shay operation. *Ind. J. Exp. Biol.,* 13: 230-233.

Alanko, J., Riutta, A., Holm, P., Mucha, J., Vapatalo, H. and Metsa-Ketela, T. (1999): Modulation of arachidonic acid metabolisms by phenol; relation to their structure and antioxidant/ prooxidant properties. *Free Radical Biology and Medicine,* 26(1-99): 193-201.

Aly, A. (1987): Prostaglandins in clinical treatment of gastroduodenal mucosal lesions: a review. *Scandinavian Journal of Gastroenterology,* 137: 43-49.

Anderson, K.N., Anderson, E..L. and Glanze, W.D. (1994): Mosby's Medical Dictionary (4^{th} ed.).Mosby, London, Boston, pp 1187-1188.

Ariyphisi, I., Toshiraru, A., Sugimura, F., Abe, M., Matsuo, Y., Honda, T. (1986): Recurrence during maintenance therapy with histamine H_2-receptors antagonist in cases of gastric ulcers. *Nation University Journal,* 28: 69-74.

Arts, J.C.W., Van de Puttee, B. and Hollman, P.C.H. (2000): Catechine contents of foods commonly consumed in the Netherlands 2. Tea, wine, fruit, juices and chocolate milk. *Journal of Agricultural and Food Chemistry*, 48: 1752-1757.

Bagchi, D., Kay, S.D., Bagchi, M., Preuss, H.G. and Stohs, S.J. (2002b): Mechanistic pathways of antioxidant cytoprotection by a novel IH636 grape seed proanthocyanidin extract. *Indian J.Exp. Biol*. 40(6): 717-726.

Bandyopadhyay, U., Biswas, K., Sengupta, A., Moitra, P., Dutta, P., Sarkar, D., Debrath, P., Ganguly, C.K., Banerjee, R.K. (2004): Clinical Studies on the effect of neem (Azadirachta indica) bark extract on gastric secretion and gastroduedenal ulcer. *Life sciences,* 75: 2867-2878.

Bastakin, S.M.A., Chandranath, I. and Garner, A. (2000): Comparison of five antisecretory agents acting via H^+-K^+ ATPase. *Journal of Physiology* (Paris), 94: 19-23.

Belaiche, J., et al., 2002.Observation Survey of NSAID-related upper gastrointestinal adverse events in Belgium. *Acta Gastroenterologic Belguim,* 65: 65-73.

Berenguer, B., Sandez, L.M., Qintez, A; Lopez-barreiro, M., Galvez, J., Martin, M.J., (2006): Protective and antioxidant effects of rhizophora Mangle L. Against NSAID-induced gastric ulcers. *Journal of Ethnopharmacology*, 103: 194-200.

Bighetti, A.E., Antonio, M.A., Kohn, L.K., Rehder, V.L.G., Foglio, M.A., Possenti, A., Vitela, L., Carvalho, J.E. (2005): Antiulcerogenic activity of a crude hydroalcolohic extract and coumarin isolated from Mikamia laevigata *Schultz Bip. Phytomedicine*, 12: 72-77.

Borong, F.W. and Boulpaep, L.E. (2005): Medical Physiology. A cellular and molecular approach (updated edition) Library of Congress *Catalogue in Publication Data*, 40: 879-907.

Borrelli, F. and Izzo, A.A. (2000): The plant kingdom as a source of antiulcer remedies. *Phytotherapy Research*, 14: 581-591.

Brian, K.B. and Turner, T.D. (1975): The practical Evaluation of phytopharmaceuticals. Wright scientific Bristol, pp 7-58.

Bruton, L.L. (1990): Agents for control of gastric acidity and treatment of peptic ulcers. In: Gilmar A.E., Rall, T.W., Neis, A.S. and Taylor, P. ed. The pharmacological basis of therapeutics (8th ed.). Pergamon Press, pp 897-913.

Bruton, L.L. (1990): Agents for control of gastric acidity and treatment of peptic ulcers. In: Gilmar A.E., Rall, T.W, Neis, A.S and Taylor, P. ed. The pharmacological basis of therapeutics, (8th ed.) Pergamon Press, pp 897-913.

Burda, S. and Oleszek, W. (2001): Antioxidant and antiradical activities of Flavonoids. *Journal of Agricultural and Food Chemistry*, 49: 2774-2779.

Burler, D.R. and Miranda, C. (2003): Antioxidant activities of flavonoids. Nutritional Research, LPI. *Exp. Med. Biol*., 472, 159-168.

Cindel, I. (1994): Methodology for the analysis of vegetable drugs: Chemical Industries Branch, Division of Industrial Operations, UNIDO, Romania, pp 24, 26 and 67.

Cody, V., Middleton, E. and Harbone, J. B. (eds.) (1986): “Plant Flavonoids in Biology and Medicine: Biochemical, Pharmaoclogical and Structure – Activity Relationships,” Alan Liss, New York.

Compete, M. (1993): Free radicals, from basic science to medicine. Poli. G. Albamo, E. and Dianzmi, M.U. (editors). Pbl. Birkhauser Verlag. Switzerland,pp: 65-79. Current Knowledge and Proposed Mechanisms: *American Journal of Physiology*, 245: 601-623.

Cook, N.C. and Samman, S. (1996): *Nutr. Biochem*, 7, 66-76.

Das, D. Bandyopadhyay, D. Bhattacharjee, M. and Banerjee, R.K. (1997): Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. *Free Radicals in Biology and Medicine*, 23: 8-18.

Davenport, H.W. (1977): Physiology of the digestive tract, 4th ed. Chap. 8, Gastric secretion U.S.A. Year Book Medical publishers incorporated, pp 42-56.

Debashis, B., Kaushu, K.B., Russel J., Reiter, T. and Ranajit, K.B. (2002): Involvement of secretive oxygen species in gastric ulceration: Protection by melatonin. *Indian, Journal of Experimental Biology,* 40: 693-705.

Decross, A. and Marshall, B. (1993): Role of *Helicobacter pylori* in Acid peptic disease. *Am. J. Med. Sci*., 306: 381-392.

Del-Valley, J. (2001): Peptic ulcer and related disorders. In: Harrison's Principles of Internal Medicine. 15th ed., Braunwald, E. Fauci, A.S. Hauser S.L., Longo D.L., Jameson J.L., (editors) Pbl McGraw-Hill, New York, p 1649.

Di Carlo, G., Mascolo, N, Izzo, A. and Capasso, F. (1997): Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sciences,* 65: 337-353.

El-Sokkary, M.B., Mansour, M.M., El-Fiky, M.S., Basyoni, M.S., El-Kotby, H., Bacyoni, M.S., Abdel-Mottaleb, A. and Okasha, M.A. (1991b): Effect of cimetidine on indomethracin-induced gastric mucosal damage and gastric secretion in rats. *J. Bio. Med. Sci. Ther,* 7(8): 109-121.

El-Sokkary, M.B., Mansour, M.M., El-Fiky, M.S., Basyoni, M.S., El-Sayed, A.Z., Abdel-Mottaleb, A.M. and Okasha, M.A.M. (1993): Relationship between indomethacin-induced gastric mucosal damage and gastric motility. *Egypt J. Med., Sci*., 14(2): 307-316.

Evans, W.C. (2000): Trease and Evans Pharmacognosy, 5th ed., W.B. Saunders, China, pp 191-394.

Falk, P. (2000): *Helicobacter pylori* – can the mechanisms of pathogenesis guidance towards novel strategies for treatment and prevention? *J. Intern. Med*. 240: 319-332.

Fan, X.G., Kellher, D., Fan, X.J., Xia, H.X. and Keeling, P.W. (1996): Helicobacter *pylori* increase proliferation of gastric epithelial cells. *GUT* 36: 19-22.

Ferreina, S.H., Moncada, S., and Vane, J.R. (1971): Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature,* 231: 237-239.

Filaretova, I., Tanaka, A., Komoike, V. and Takeuchi, K. (2002): Selective COX-2 inhibitor induces gastric mucosal damage in rats with adrenalectomy (Abstract). *Gastroenterology,* 122: A516.

Ford, A.C., Delaney, B.C., Formann, D. and Moayyedi, P. (2004): Eradication therapy in helicobacter pylori positive peptic ulcer disease: systemic review and economic analysis. *Am J. Gastroenterology,* 99(9): 1833-65.

Ford, A.C., Delaney, B.C., Formann, d. and Moayyedi, P. (2004): Eradication therapy in helicobacter pylori positive peptic ulcer disease: System review and economic analysis. *Am J. Gastroenterology*, 99(9): 1833-65.

Form, D.M. and Auerbach, R. (1983): PGE2 and angiogenesis. *Proc. Soc. Exp. Biol. Med,* 172: 214-8.

Forman, D., Newell, D.G., Fullerton, F., Yarnell, J.W., Stacey, A.R., Wald, N. and Silas, F. (1991): Association between infection with helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation: *British Medical Journal* 302: 1302-1305.

Fries, J.T. (1991): NSAIDs gastropathy: The second most deadly rheumatic disease? Epidemiology and risk Appraisal. *J. Rheumatol,* 28 (Suppl.): 6-10.

Fuleki, T. and Ricardo de Silva, J.M. (1997): Catechin and procyanidins composition of seds from grape cultivars grown in Ontario. *Journal of Agricultural and Food Chemistry,* 45: 1156-1160.

Gaginella, T.S., Muller, R.K., Witt, C.G., Rosen, P., Holland, G.W., Gallotorres, H. and Sulivan, A.C. (1985): Inhibition of acid secretion and gastric lesions in rats by the prostanoid RO22-6923. *J. Pharmacol. Exp. Ther.,* 232 (1): 202-207.

Ganong, W.F. (2005): Review of Medical Physiology, Twenty-second edition: Lange Publication, USA, pp 467-477.

Garner, A. (1992): Adaptation in the pharmaceutical industry with particular reference to gastrointestinal drugs and diseases. *Review Scandinavian Journal of Gastroenterology,* 193: 583-589.

Ginner, E., Hagymasi, K., Blazonics, A., Kery, A., Szoke, E. and Lembekorics, E. (2001): The in vitro effects of Helichysi floss on microsomal lipid peroxidation. *Journal of Ethnopharmacology,* 77: 31-35.

Glavia, G.B. and Mikhail, A.A. (1976): Stress and ulcer etiology in the rat. *Physiol. Behav,* 16: 135-9.

Goel, R.K. and Sairam, K. (2002): Antiulcer drugs from indigenous sources with emphasis on Musa sapientum. Tamrabhasnia. Asparagus racemosus and zingiber officinale. *Indian Journal of Pharmacology,* 345: 100-110.

Gonzalez, F., Portela, T., Stipp, E. and Distasi, L. (2001): Antiulcerogenic and analgesic effects of Nyterus aqm folium, sorocea blomplandii and zolerima ilicifolia, *Journal of Ethnopharmacology,* 106: 57-61.

Graham, D.Y. (1996): No-steroidal anti-inflammatory drugs, helicobacter pylori and ulcers: where we stand. *Am J. Gastroenterol.* 91(10): 2086.

Grossman, M.I. (1973): In physiology for the physicians. *A. Monthly publication of American Physiological Society,* vol. 1(7): 1-5.

Grower, J.K. and Yadav, S.P. (2004): Pharmacological actions and potential uses of Momordica charantia: a review. *Journal of Ethnopharmacology,* 93: 123-132.

Guyton, A.C. and Hall, J.E. (2006): General principles of gastrointestinal function. In: Textbook of Medical Physiology. Eleventh ed. USA, Saunders Publishers, pp 718-726.

Halter, F., Schmassmann, A. and Peskar, B.M. (2001): Cyclooxygenase – 2 implications on maintenance of gastric mucosal integrity and ulcer healing: Controversial issues and perspectives, *GUT,* 49: 448-453.

Hammerstone, J.F., Lazarus, S.A. and Schmitz, H.H. (2000): Procyanidin content and variation in some commonly consumed foods. *Journal of Nutrition,* 130: 2086S-2092S.

Hano, J., Bogajske, J., Danek , L. and Wantuch, C. (1976): The effect of neuroelptics on the development of gastric ulcers in rats exposed to restraint cold stress. *Pol. J. Pharma. C., Pharm*: 28: 37-47.

Harborne, J.B. and Williams, C.A. (2000): *Phytochemistry,* 55: 481-504.

Hawkey, C.J. (2000): Nonsteroidal anti-inflammatory drug gastropathy: causes and treatment. *Scand J. Gastroenterol*, 31 (*Suppl*. 220): 124-127, 34.

Heinrid, M. (2003): Ethnobotany and natural products: the search for new molecules, new treatments of old diseases or a better understanding of indigenous cultures. *Current Topics in Medical Chemistry,* 3: 141-154.

Higham, J., Kang, J.Y. and Majeed, A. (2002): Recent trends in admissions and mortality due to peptic ulcer in England. Increasing frequency of haemorrhage among older subjects, *GUT,* 50: 460-464.

Hoogerwerf, W.A. and Pasricha, P.J. (2001): Agents used for the control of gastric acidity and treatment of peptic ulcers and gastrooestopahgeal reflux diseases. In: Hardman, J.G., Limbrid, L.E., Goodman, C.C., Cilman, B.T (eds.). Goodman and Gilman's: The Pharmacological Basis of Therapeutics. 10^{th} ed. McGraw-Hill, New York, pp 1005-1019.

Huang, J.Q., Sridhar, S. and Hunt, R.H. (2002): Role of helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet*, 359: 14-22.

Hughes, R. (1993): Diagnosis and treatment of gastric polyps gastrointestinal endoscopy clin research. *Am J*, 2: 457-467.

Kato, S., Tanaka, T., Mizoguchi, H. and Takeuchi, K. (2000): The role of nitric oxide and prostaglandins in alterations of ulcerogenic and healing responses in adjuvant-induced arthritic rat stomachs, *Alimentary Pharmacology and Therapeutics*, 14(*Suppl*. 1): 18-25.

Kawaguchi, H., Pilbeam, C.C., Gronowicz, G., Fletcher, B.S. and Herschman, H.R. (1995): Transcriptional induction of prostaglandin G/H synthetase-2 by basic fibroblast growth factor. *J. Clin. Invest*. 96: 923-30.

Keldman, M. and Colturi, T.J. (1984): Effect of indomethacin on gastric acid and bicarbonate secretion in humans. *Gastroenterology*, 87: 1339-1343.

Khayyala, M.T., Seif-El-Nasra, M., El-Ghazalyb, M.A., Okpanyic, S.N., Kelberc, O. and Weisere, D. (2006): Mechanisms involved in the gastro-protective effect of STW 5 (Iberogasts) and its components against ulcers and rebound acidity. *Phytomedicine* 13 SV 56-66.

Kikkawa, A., Iwakiri, R. and Ooctani, H. (2005): Prevention of the Re-haemorrhage of bleeding peptic ulcers: Effect of helicobacter eradication and acid suppression. Aliment pharmacological therapy, 2: 79-84.

Kim, D.O. and Lee, C.Y. (2004): Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Critical Review Food Science and Nutrition*, 44(4): 253-273.

Kontarek, S.J., Radeccki, T., Brzozowsky, T., Drozdowicz, D., Piastuchi, I., Muramatsu M., Tanaka M. and Aihara H. (1986): *Eur. J. Pharmacol*, 125: 185-192.

Koyabashi, K., Arakawa, T., Satoh, H., Fukudo, T. and Nakamura, H. (1985): Effects of indomethacin, tiaprofenic acid and diclofenac on rat gastric mucosal damage and content of prostacyclin and prostaglandin F_2, Prostaglandins, 30(4): 609-618. 101:75-7.

La Casa. C., Villegas, I., Alarc on de la Lastra, C., Motilva, V. and Calero, M.J.M. (2000): Evidence for protective and antioxidant properties of rutin, a natural flavone against ethanolinduced gastric lesions. *Journal of Agricultural and Food Chemistry,* 49: 3341-3348.

Laujy, Sung J.J. and Lee, K.K. (2000): Effect of intravenous omeprazole on recurrent bleeding after endoscopic treatment of bleeding peptic ulcers. *N. Engl. J. Med.* 343(5): 310-6.

Laujy, Sung J.J. and Lee, K.K (2000): Effect of intravenous omeprazole on recurrent bleeding after endoscopic treatment of bleeding peptic ulcers. *N. Engl. J. Med.* 343 (5): 310-6.

Lewis, D.A., Fields, W.N. and Shaw, G.P.J. (1999): *Ethnopharmacol*, 65, 283-288.

Lim, J.W., Kim, H. and Kim, K.H. (2001): NF-Kappa^{B}, inducible nitric oxide synthase and apoptosis by helicobacter pylori infection. *Free Radical Biology and Medicine,* 31: 355-366.

Lin, H.J, Lowe, C. and Lee, F.Y. (1998): A prospective randomized comparative trial showing that omeprazole prevent rebleeding in patients with bleeding peptic ulcer after successful endoscopic therapy. *Arch Intern Med*, 158(1): 54-8.

Lin, H.J., Lower, C, Lee, F.Y. (1998): A prospective randomized comparative trial showing that omeprazole prevent rebleeding in patients with bleeding peptic ulcer after successful endoscopic therapy. *Arch Intern Med*. 158 (1): 54-58.

Lovke, D. (1983): A new approach to practical acute toxicity testing. *Arch. Toxicol*, 53: 275-287.

Madden, R.J., Ramsburg, H.H. and Hundley, J.M. (1951): A critical study of the Shay rat. *Gastroenterology*; 18:119-127.

Maia, I.H. and Whittle, B.J. (1975): Investigation of the vasodilator and antisecretory role of prostaglandins in the rat gastric mucosa by use of nonsteroidal anti-inflammatory drugs. *Brit. Jour. Pharmacol,* 53: 217-24.

Maiden, L.P. and Harris, A.W. (2002): Lansoprazole for the prevention of recurrences of ulcer complications from long-term low dose aspirin. *N. Engl. J.M,* 347(202): 1623-4.

Martin, M.J., LaCasa, C., Alarcon-de-Lastra, C., Cabeza J., Villegas, I. and Moltiva, V., (1998): Antioxidant mechanisms involved in gastroprotective effects of quercetin Zeistschriff fur Naturforschung C, 52: 82-88.

Mequanente, S., Markonnen, E., Debella, A. (2006): Gastric acid antisecretory and acid neutralization effect of aqueous Trigenella fenoum-gracum and linum ussitatissimum seed extracts on experimental models. *Pharmacology on line,* 3: 192-200.

Merki, H.S. and Wildersmith, C.H. (1994): Do continuous infusion of omeprazole and ranitidine retain their effect with prolonged dosing? *Gastroenterology* 106(1): 60-64.

Mersereauu, W.A. and Hinchey, E.J. (1982): Prevention of phenylbutazone ulcer in the rat by glucose role of a glycoprivic receptor system. *Am J. Physiol Gastrointest. Liver Physiol,* 242: G429-G432.

Mertz, D., Pratha, V. and Martin, P. (2000): Oral and intravenous dosage forms of pantoprazole are equilvalent in their ability to suppress gastric acid secretion in patients with gastroesophageal reflux disease. *Am J. Gastroenterology,* 95(3): 26-33.

Miller, J.P. and Faragher, E.B. (1986): Relapse of duoduenal ulcer: does it matter which drug is used in initial treatment? *British Medical Journal,* 293: 1117-1118.

Moore, K.L. (1991): Clinically Oriented Anatomy 7th ed. Lippincott Williams and Wilkins, 160-164.

Moore, K.L. and Dalley, A.F. (2006): Clinically oriented Anatomy 5th ed. Lippincott Williams and Wilkins, pp 252-257.

Narkhund, S.L. (1982): Natural antioxidant enzymes. *Cancer Research*: 42(5): 1955-1961.

National Institutes of Health (2002): National Digestive Disease Information Clearing House: Helicobacter pylori and peptic ulcer disease.

Negrini, R., Savio, A., Poiesi, C., Appelmilk, B.J., Buffoli, F., Paterlini, A., Oesari, P., Graffeo, M., Vaira, D. and Franzin, G. (1996): Antigenic mimickry between helicobacter pylori and gastric mucosa in the pathogenesis of body atrophic gastritis. *Gastroenterology,* 111: 655-665.

O'Malley, P. (2003): Gastric ulcers and Gerd. The new "plagues" of the 21st century update for the clinical nurse specialist. *Clinical Nurse Specialist* 17: 286-289.

Okabe, S. , Honda, K., Takeuchi, K. and Takagi, K. (1975): Inhibitory effect of L-glutamine on gastric irritation and back-diffusion of gastric acid in response to aspirin in rat. *J. Dig. Dis,* 20 (7): 626-631.

Okabe, S., Takeuchi, K., Urushidani, T. and Takagi, K. (1977): Effects of cimeditine, a histamine H_2-receptor antagonist on various experimental gastric and duodenal ulcers. *Am. J.Dig. Dis,* 22: 667-684.

Parsonnet, J., Friedman, G.D., Vandersteen, D.P., Chang, Y., Vogelman, J.H., Orentreich, N. and Sibley, R.K. (1991): Helicobacter pylori infection and the risk of gastric carcinoma. *The New England Journal of Medicine*, 325: 1127-1131.

Pavo, J., Morschl, E., Szepes, Z., Kiss, J., Boda, K., Vetro, G., Varga C., Laszlo, F. (2000): Vasopressin deficiency decreases the frequency of duodenal ulceration in man. *Journal of Physiology,* (Paris) 94: 63-66.

Peskar, B.M. and Maricic, N. (1998): Role of prostaglandins in gastroprotection. *Digestion Disease Science,* 43: 235-295.

Phull, P.S., Green, C.J. and Jacyna, M.R. (1995): A radical review of stomach: The role of oxygen-derived free radicals in gastroduodenal disease. *American Journal of Gastroenterology and Hepatology*, 7: 265-274.

Rainsford, K.D. (1987): The effect of 5-lipoxygenase inhibitors and leukotrienes antagonists on the development of gastric lesions induced by NSAIDS in mice. *Agents and Action,* 21: 316-319.

Ramirez, R.O. and Rao, .J.R. (2003): The gastro-protective effect of tannin extract from Duhar (Syzgium cumini skeels) bark on HCl/ethanol induced gastric mucosal injury in rat. *Clin Hemorrhagic Microcirc,* 79(3-4): 253-61.

Rao, V., Ojha, S.K., Radhakrishnan, K., Govindarajan, R., Rastogi, S., Merhrotra, S., Pushpangadan, P. (2004): Antiulcer activity of utleria salicifolia rhizome extract. *Journal of Ethnopharmacology,* 91: 243-249.

Rao, V.S., Santos, F.A., Sobeira, T.T., Souza, M.F., Melo, C.L. and Silveira, E.R. (1997): Investigations on the gastroprotectie and antidiarrhoeal properties of termatin, a tetramethroxy-flavone from Egletes viscose. *Plant Med*, 63(20): 146-149.

Rave, D. (1999): Helicobacter pylori: Epidemiology and pathogenesis in Barndt, L., Daum, F., Friedman, L., et al. (eds). Clinical practice of Gastroenterology, Philadelphia, *Current Medicine Inc*., 249-254.

Raymond, J., Tania, M., Rebecca, C. and Ruth, E. (1995): Human spasmolytic polypeptide is a cytoprotectie agent that stimulates cell migration. *Gastroenterology*, 108: 108-116.

Repetto, M.G. and Liesuy, S.F. (2002): Antioxidant properties of natural compounds used in popular medicine for gastric ulcer. *Brazilian Journal of Medical and Biological Research,* 35: 523-534.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. (1996): Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20: 933-956.

Robert, A., Nezamis, J. and Phillips, J. (1968): Effect of prostaglandin EI on gastric secretion and ulcer formation ion the rat. *Gastroenterology,* 55: 481-7.

Robert, A. and Nezamis, J.E. (1964): Histopathology of steroid-induced ulcers. *Arch. Pathol.*, 77: 407-423.

Ronke, B., Jones, N. and Sherman, P. (1996): *Helicobacter pylori* infection and peptic ulcer disease in children. Pediatr. *Infect. Dis. J.* 15: 1-13.

Roth, S.H., Bennet, R.E., Mitchell, C.S. and Hartman, R.J. (1987): Cimetidine therapy in nonsteroidal anti-inflammatory drug gastropathy. *Arch. Intern. Med*, 147: 1798-1801.

Sacchetti, G., Medici, A., Maietti, S., Radice, M., Muzzoli, M., Manfredini, S., Braccioli, E. and Bruni, R. (2004): Composition and functional properties of the essential oil of Amazonian basil, Ocimum micranthum Wild, Labiatae in comparison with commercial essential oils. *Journal of Agricultural and Food Chemistry*, 52: 3486-3491.

Sato, N., Ouan, L.H., Kawano, S., Kamada, T. and Abe, H. (1984): Effects of H_2 receptor and muscarine receptor antagonists and prostaglandin E., analogue on the gastric mucosal hemodynamics and oxygen sufficiency in rats. *Adv. Exp. Med*, 180: 763-771.

Sattyavathin, G.V., Gupta, A.K. and Tandon, N. (1967): Medicinal Plants of India, vol. 2 ICMR, New Delhi, p. 574.

Scepovic, Z. and Radmanovic, B.Z. (1984): Interaction between roserpine and nonsteroidal anti-inflammatory agents in producing gastric ulcers in rats. *Europ. J. Pharmacol,* 28: 445-448.

Sembulingam, K. and Sembulingam, P. (2003): Inhibition of acid secretion. *Essentials of Medical Physiology*, 2: 169-171.

Sembulingam, K. and Sembulingam, P. (2005): Gastrointestinal Physiology. In: Essentials of Medical Physiology. (Third Edition) India, Jaypee Brothers Medical Publishers; 176-185.

Sharma, M.P. and Ahuja, V. (2003): Current Management of Acid Peptic Disorders.. Review article, *Indian Academy of Clinical Medicine.*

Shay, H., Komarov, S., Fells, S.S. Meranz, D., Gruenlein, M. and Siplet, H. (1954): A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology,* 5: 43-61.

Shidani, A. and Graham, D.Y. (2002): Pathogenesis and therapy of gastric and duodenal ulcer disease. Medical clinical North America, 86: 1447-1466.

Shiodam, A. and Graham, D.Y. (2002). Pathogenesis and therapy of gastric and duodenal ulcer disease. *Medical clinical North America* 86, 1447-1466.

Soll, A.H. (1996): Consensus conference, Medical Treatment of Peptic Ulcer disease. Practical guidelines. Practice Parameters Committee of the American College of Gastroenterology. *The Journal of the American Medical Association*, 275: 622-629.

Soll, A.H., Kurata, J. and McGuigan, J.E. (1989): Ulcers, nonsteroidal anti-inflammatory drugs and related matters. *Gastroenterology*, 96 (2 pt 2): 561-568.

Stables, R., Campbell, C.., Clayton, N.M., Clitherow, J.W., Grinham, C.J. McColm, A.A. McClarent, A. and Trevethick, M.A. (1993): Gastric anti-secretory, mucosal protective, anti-pepsin and anti-*helicobacter* properties of ranitidine, bismuth citrate. *Aliment Pharmacol Ther*, 7: 237-246.

Takase, H., Yamamoto, K., Hirano, H., Saito, Y. and Yamashita, A. (1994): Pharmacological profile of gastric mucosal protection by marmin and nobiletin from a traditional herbal medicine, Auranti fructus immature. *Japanese Journal of Pharmacology,* 66: 139-147.

Takeuchi, K., Ueki, S. and Okabe, S. (1986): Importance of gastric motility in the pathogenesis of indomethacin-induced gastric lesions in rats. *Dig. Dis. Sci,* 31: 1114-1122.

To, K.F., Chan, F.K.L., Chen, A.S.L., Lee, T.L., Ng, Y.P. and Sung, J.J.Y. (2001): Up-regulation of Cyclooxygenase-1 and -2 in human gastric ulcer. *Alimentary pharmacology and therapeutics,* 15: 25-34.

Toma, W., Hiruma-Lima, C.A., Guerrero, R.O., Brito, A.R.2005. Preliminary studies of Mammea Americana L. (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice. *Phytomedicine,* 12: 345-350.

Ueki, S., Takeuchi, K. and Okabe, S. (1988): Gastric motility is an important factor in the pathogenesis of indomethacin-induced gastric mucosal lesion in rats. *Dig Dis Sci,* 33(22): 209-216.

Urushidani, T., Kasuya U, and Okabe, S. (1979): The mechanism of aggravation of indomethacin-induced gastric ulcers by adrenalectomy in the rat. *Jpn. J. Pharmacol*, 29:775-780.

Vane, J.R. (1971): Inhibition of prostaglandin synthesis as a Mechanism of action of aspirin-like drugs. *Nature New Biology* (London) 231: 232-235.

Vergara M, Catalan M, Gusbert JP, Calvet X. (2005): Meta-analysis, role of helicobacter pylori eradication in the prevention of peptic ulcer in NSAID users. *Aliment pharmacological therapy*, 21(12): 1411-8.

Vergara, M., Catalan M., Gusbert, J.P. and Calvet, X. (2005): Meta-analysis, role of helicobacter pylori eradication in the prevention of peptic ulcer in NSAID users. *Aliment Pharmacological Therapy*, 21(12): 1411-8.

Villar, A., Gasco M.A. and Alcaraz, M.J.J. (1984): *Pharm. Pharmacol*, 36, 820-823.

Voutianen M, Mantynen, T., Farkkila, M. (2001): Impact of nonsteroidal anti-inflammatory drug and aspirin use on the prevalence of dyspepsia and uncomplicated peptic ulcer disease. *Scand. J Gastroenterol*, 36: 81-821.

Wallace, J. 2001. Mechanisms of protection and healing. Current knowledge and future research. *American Journal of Medicine*, 110: 195-225.

Wallace, J.L. (2001): Mechanisms of protection and healing: Current knowledge and future research. *American Journal of Medicine,* 110: 19S-22S.

Wallace, J.L., Granger, D.N. (1991): .Pathogenesis of NSAID gastropathy: are neutrophils the culprits? *Trends in pharmacological sciences,* 13: 129-131.

Wallace, J.L., McKnight, W., Reuter, B.K., Vergnolle, N. (2000): NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* 119: 706-714.

Way, W. (1981): Anatomy of the rat stomach. *Gastroenterology*, 61: 413-414.

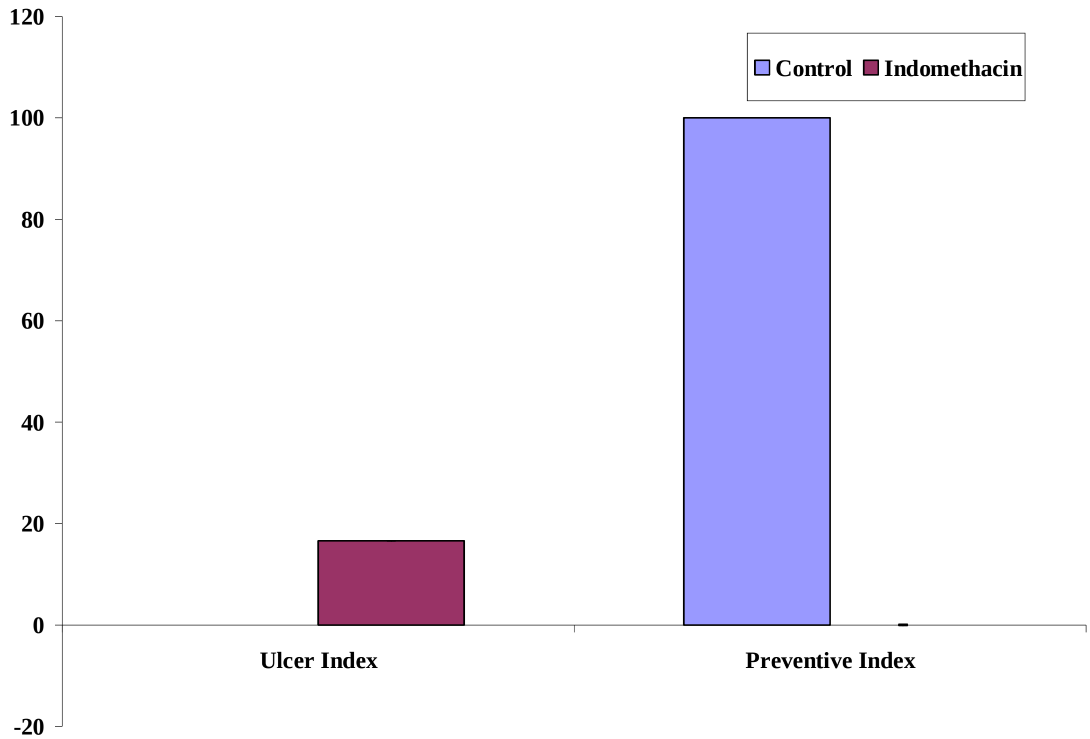
Weeks, D., Eskanri, S. and Scott, D. (2000): AH^+ - *Helicobacter Pylori*-gated urea channel: the link between *helicobacter pylori* urease and gastric colonization. Science, 287: 482-485.

Wei, Y.H. (2002): Chemistry and Biochemistry of free radical Antioxidant. *Experimental Biology and Medicine*, 227: 674-662.

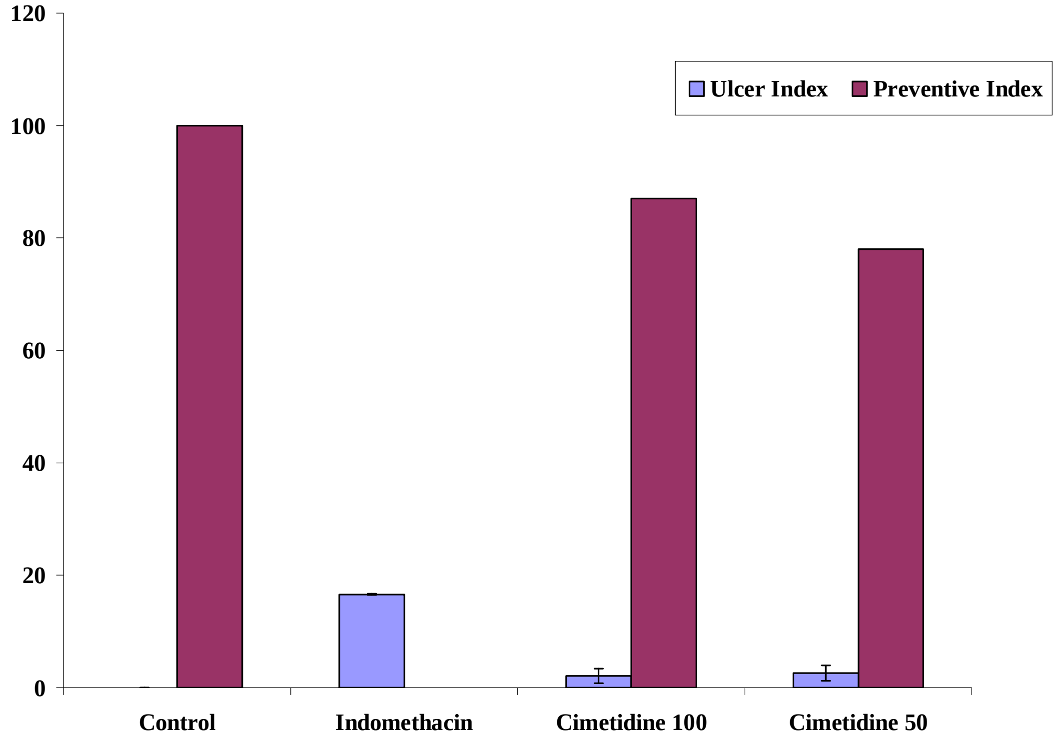
Yao, X and Forte, J.G. (2003): Cell biology of acid secretion by the parietal cells. *Annu. Rev. Physiol*, 65: 103-131.

Yesilada, E., Sezik, E., Fujita, T., Tanaka, S. and Tabata, M., (1993): Screening of some Turkish medicinal plants for their antiulcerogenic activities. *Phytotherapy Research*, 7: 263-265.

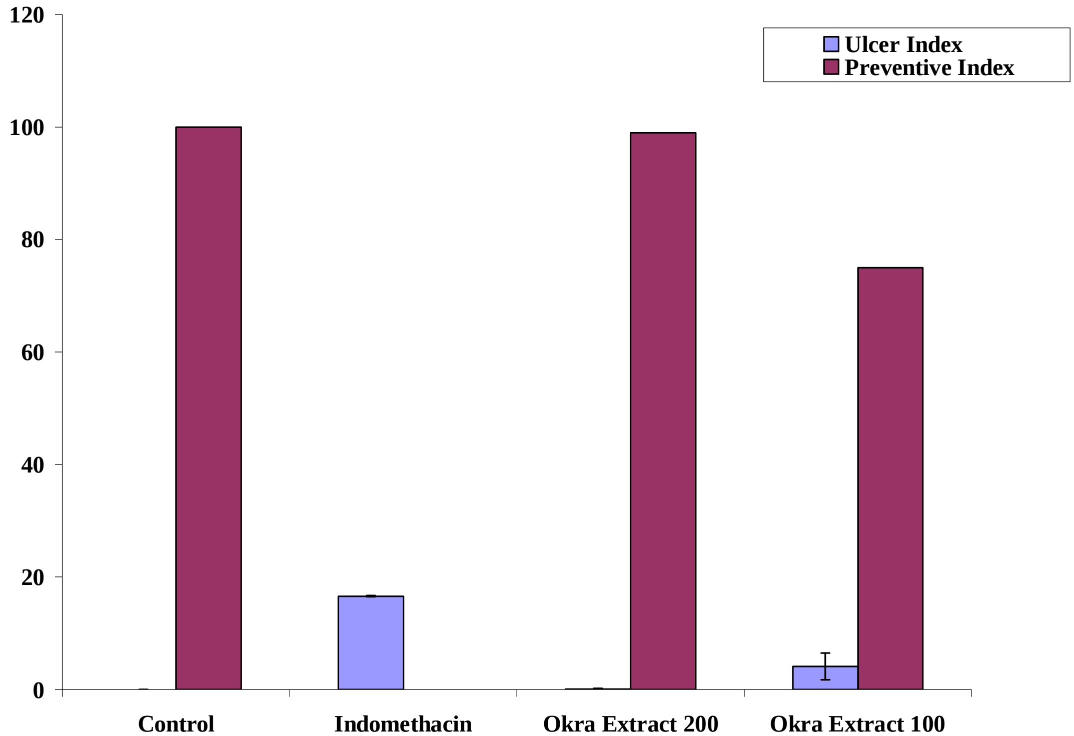
APPENDICES



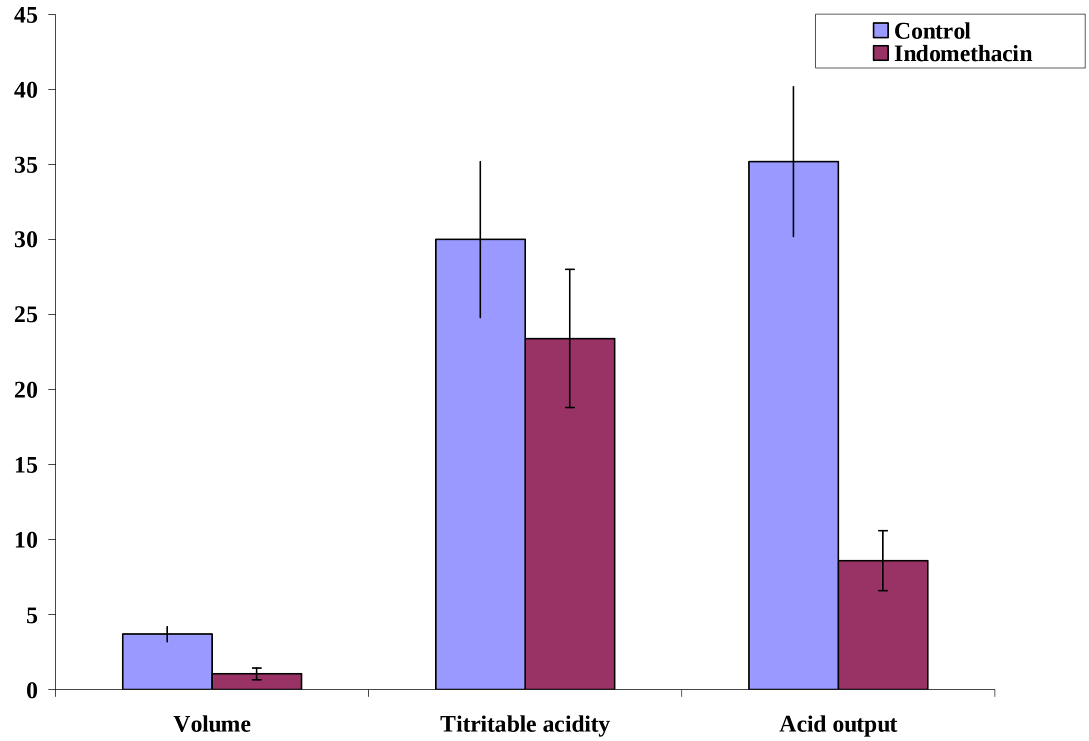
Appendix 1: **Ulcer Index and preventive index for control and indomethacin-treated rats**



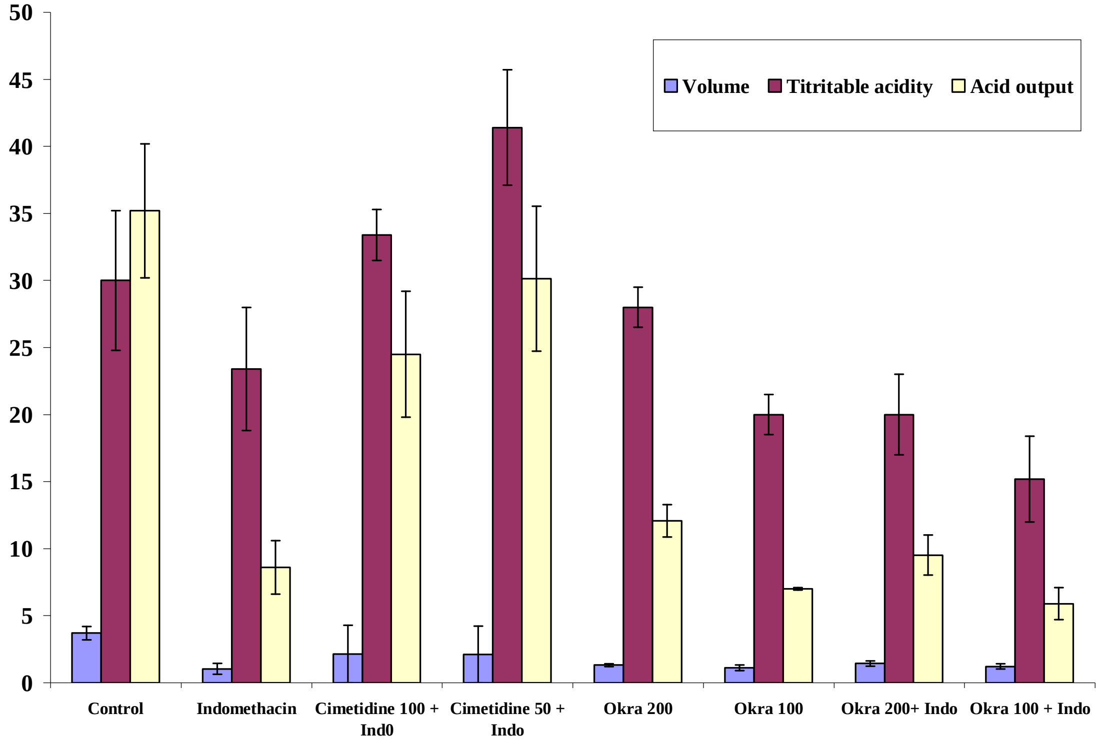
Appendix II: Ulcer Index and preventive index for control, indomethacin and cimetidine-treated rats



Appendix III: **Ulcer Index and preventive index for control, indomethacin and Okra extract-treated rats**



Appendix IV: Parameters of gastric secretion at 3 hours for control and indomethacin-treated rats



Appendix V: Parameters of gastric secretion at 3 hours for control, indomethacin, Okra extract and cimetidine-treated rats