STUDY ON THE GASTRO-PROTECTIVE EFFECT OF PARTIALLY PURIFIED ROOT EXTRACTS OF *Ziziphus abyssinica* IN ETHANOL INDUCED ULCER IN RATS

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AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

OCTOBER, 2015

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MSc/SCI/31131/12-13

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,

AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD

OF A

MASTER OF SCIENCE DEGREE IN BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY,

FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

OCTOBER, 2015

DECLARATION

I declare that the work in this dissertation entitled "Study on the gastro-protective effect of partially purified root extracts of *Ziziphus abyssinica* in ethanol induced ulcer in rats" has been carried out by me in the Department of Biochemistry. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

SALAHUDDEEN YAU		
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled STUDY ON THE GASTRO-PROTECTIVE EFFECT OF PARTIALLY PURIFIED ROOT EXTRACTS OF *Ziziphus abyssinica* IN ETHANOL INDUCED ULCER IN RATS by Salahuddeen YAU meets the regulations governing the award of a master degree in Biochemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This research work is dedicated to the entire family of Alhaji Ya'u Garba Faskari.

ACKNOWLEDGEMENT

All praises and glorifications are due to Almighty Allah for His infinite mercies, guidance, love and protection, who gave me the strength and capacity to carry out this thesis successfully. Through His help, I have been able to attain this level.

My infinite respect and profound gratitude goes to my supervisors; Prof. K.M. Anigo and Dr. M.A. Ibrahim, who out of their vast knowledge and experiences rendered their advices, suggestions and guidance towards entire completion of this research. I ask Allah, the Lord of the worlds to accept this humble effort of yours and add it to your records in the day of judgment when neither child nor wealth will avail, except he who comes to Allah with a sound heart.

My sincere gratitude also goes to Prof. I.A. Umar (Head of department) and the entire staff of the department for their great generosity in sharing their knowledge of Biochemistry with us. I really appreciate your contribution and in fact it enhanced our understanding and ability to reason and respond scientifically. May Almighty Allah increase your knowledge and wisdom, amen.

My greatest commendation and appreciation to my parents; Alhaji Yau Garba Faskari and Hajiya Hauwau Maharazu, who toiled day and night for realization of my academic pursuit. May Allah spare our lives in good health and grant you His highest garden (Firdaws) amen. Education is the greatest asset one can ever acquire. This was possible through your care, love encouragement, financial and moral support.

I appreciate the love, encouragement, advice, prayers and concern shown by my dear wife Hajiya Aishat Umar, I pray may Allah bless our children and reward you with His firdaws, amen.

To my brothers and sisters; Abba (Abubakar), Nafiu, Yusuf, Maharazu , Ikilima, Abdulmalik and others I could not mention here, may our struggles have fruitful end, amen.

Deserve mentioning are my intimate friends; Sabiu Mudassir, Sagir Bello, Aliyu Bara'u, Usman Idris, Yusuf Bature, Ibrahim Mahmoud, Umar Hamza, Abubakr Abdullahi and others numerous to mention. May Allah bless you all.

My unreserved appreciation to my colleagues whom we started this struggles together; Abdullahi Salisu, Aliyu Yakubu, Muhammad Abubakar Song, Muhammad Labaran Kano, and Jiyil Markus. I appreciate your love and concern during the period of our stay in Zaria. May our lives be blissful.

I can't forget the guidance and help rendered to me by Dr. Auwal Garba, Aliyu Mansir Ikara, and Nura Giwa during my stay in A.B.U. May Allah grant you the best in this world and hereafter.

ABSTRACT

Aqueous, Methanol, Ethyl acetate and Hexane roots extracts of *Ziziphus abyssinica* were investigated for their effects on gastric lesion in rats administered with1ml of 95% ethanol and the antiulcer activity of *Ziziphus abyssinica* was compared to that of control. Pre-treatment of animals with the extracts (300mg/kg and 600mg/kg) orally once daily for two weeks significantly ($p<0.05$) reduced the formation of ulcers induced by ethanol, with aqueous extract having the highest effect. Percentage inhibition was 95% and 93%. The hexane extract afforded the least protection with 23.8% and 28.6%. The aqueous extract was further fractionated with hexane, ethyl acetate and butanol. The yields were 0g, 0.76g, 21.3g respectively and 37.4g for the remaining aqueous fractions. The aqueous fractions were found to significantly reduced ulcer index, percentage inhibition being 52.6 and 57.1% while the butanol has 28.6 and 18.1% respectively. The volume of the gastric juice and gastric acidity was significantly decreased as compared to control group, with significant increase in pH. Also, glutathione concentration, superoxide dismutase and catalase activities were maintained in *Z. abyssinica* root fractions administered animals. From the column chromatography, four fractions were obtained and each was tested for antiulcer effect. Fraction C showed highest antiulcer effect. The results indicate that *Ziziphus abyssinica* root extracts possess antiulcer effect with aqueous extract and aqueous fractions having the highest protective effect. The gastro-protective effect of *Z. abyssinica* roots could therefore be due to its cytoprotective (increased mucus secretion), antisecretory (decreased gastric juice volume, decreased acidity levels and increased pH) and antioxidant properties.

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Table of Contents

Page

Cover page……..... i

Fly leaf ……..... ii

Title page……..... iii

Declaration……..... iv

Certification……..... v

Dedication..... vi

Acknowledgements……..... vii

Abstract……..... ix

Table of Contents…….....x

List of Figures……..... xvii

List of Plates……..... xviii

List of Tables…….. ixx

Abbreviations……..... xx

1.0 **INTRODUCTION**……..... 1

1.1 Introduction……..... 1

1.2 Statement of Research Problem……..... 3

1.3 Justification…….....4

1.4 Aim and Objectives…….....5

1.5 Hypothesis..... 5

2.0 **LITERATURE REVIEW**……..... 6

2.1 **History of Medicinal Plants**……..... 6

2.1.1 Significance of *Ziziphus abyssinica* in Ethnic Folk Medicine…….. 8

2.1.2 Morphology and Geographical Distribution of *Ziziphus abyssinica*…… 8

2.1.3 Scientific Classification of *Ziziphus abyssinica*…… 9

2.1.4 Biological effects of *Ziziphus abyssinica***…….** 9

2.2. **Overview of Ulcer Pathophysiology**……. 11

2.2.1. **Causes of Peptic Ulcer**…… 12

2.2.1.1Non-steroidal Anti-inflammatory Drugs……..…. 12

2.2.1.2 Stress……..…..... 13

2.2.1.3Mechanism of Ulcer induction Ethanol……. 14

2.2.1.4 *Helicobacter pylori*…….. 15

2.2.2 **Cytoprotective Factors for Ulcer**……...17

2.2.2.1 Gastric Mucus…….. 17

2.2.2.2 Prostaglandins…….. 18

2.2.2.3 Antioxidants……. 19

2.3 **Drugs used in the Management of Ulcer..**…… 20

2.3.1 Histamine Receptor Antagonists…… 20

2.3.2 Proton Pump Inhibitors……..…….. 22

2.3.3 Muscarinic Receptor Antagonists……..……..... 22

2.3.4 Antacids…….……....23

2.4 **Some Plant Secondary Metabolites That Protect From Ulcer**…….**.....**23

2.4.1 Flavonoids……..... 23

2.4.2 Tannins……..…….**.....** 24

2.4.3 Alkaloids……..…… 26

3.0 **MATERIALS AND METHODS**…….. 28

3.1 **Materials**……28

3.1.1 Chemicals/Reagents…….……28

3.1.2 Experimental animals**.....** 28

3.1.3 Plant Material Collection……..…… 28

3.2 **Methods**…… 29

3.2.1 Preparation of the extracts…… 29

3.2.2 Antiulcerogenic Study of the extracts……. 29

3.2.3 Gastric Ulcer Induction……31

3.2.4 Ulcer Index……. 31

3.2.5 Percentage Inhibition…… 31

3.2.6 LD50 Determination of Aqueous extract…… 32

3.2.7 Fractionation of the Most Active extract…….. 32

3.2.8 **Antiulcerogenic study of butanol and aqueous fractions**…… 32

3.2.8.1 Animal Grouping…… 32

3.2.8.2 Collection of Sample…… 33

3.2.9 **Determination of Gastric Ulcer Parameters**…… .33

3.2.9.1 Determination of Gastric juice volume…….……..33

3.2.9.2 Determination of gastric juice pH……. 34

3.3.0 **Biochemical Analyses**…… 34

3.3.1 Estimation of Total and Free acidity…… .34

3.3.2 Estimation of Gastric Wall Mucus……34

3.3.3 Superoxide Dismutase Assay……35

3.3.4 Catalase Activity Determination…… 36

3.3.5 Glutathione Concentration……36

3.4.0 **Purification of Crude Aqueous Fraction**…….. 37

3.4.1 Thin Layer Chromatography…… 37

3.4.2 Column Chromatography…… 38

3.5.0 **Antiulcerogenic Study of the Fractions from Column Chromatography**..38

3.6 Histological Studies…… 39

3.7 Statistical Analysis…… 39

4.0 **RESULTS**……..... .40

4.1 Effect of *Z. abyssinica* root extracts on lesion index…… 40

4.2 Determination of LD_{50} of Aqueous Extract……. 40

4.3 Antiulcer activity of Aqueous and Butanol Fractions……..45

4.4 Column Chromatography…….. 51

4.5 Effect of *Z. abyssinica* root column fractions on gastric secretion parameters.. 53

5.0 **DISCUSSION**…… 56

5.1 Discussion……... 56

6.0 **SUMMARY, CONCLUSION AND RECOMMENDATIONS**…… 60

6.1 Summary……..60

6.2 Conclusion…… 61

6.3 Recommendations…… 62

REFERENCES…… 63

APPENDICES……... 73

LIST OF PLATES

I *Ziziphus abyssinica*…….. 10

II Photomicrographs of Stomach Tissue of Rats Pretreated with Extracts of Roots *Ziziphus abyssinica* and Cimetidine…… 44

III Photomicrographs of Stomach Tissue of Rats Pretreated with Aqueous And Butanol Fractions……. 49

IV Photomicrographs of Stomach Tissue of Rats Pretreated with Fractions From Column Chromatography…… 55

LIST OF FIGURES

Fig. 4.1 Percentage protection of *Z. abyssinica* extracts…… 43

Fig. 4.2 Effect of *Z. abyssinica* fractions on mucus content of control and experimental animals…….. 48

Fig. 4.3 Effect of *Z. abyssinica* root fractions on catalase, superoxide dismutase activities and glutathione concentration…… 50

Fig. 4.4 TLC profile of aqueous fraction…… 52

LIST OF TABLES

Table 2.1 History of Natural Product medicine..... 6

Table 4.1 Effect of *Ziziphus abyssinica* root extracts on gastric lesion Index..... 42

Table 4.3 Effect of Aqueous and butanol fractions on Gastric Secretion Parameters 47

Table 4.4 TLC Properties of Column Fractions from *Z. abyssinica* roots..... 51

Table 4.5 Effect of *Ziziphus abyssinica* root column fractions on Gastric Secretion Parameters..... 54

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
b.w.	Body weight
CAT	Catalase
CC	Column Chromatography
COX	Cycloxygenase
COX-1	Cycloxygenase-1
COX-2	Cycloxygenase-2
DTNB	5,5-dithionitroBenzoic acid
GGT	Gammaglutamyltranspeptidase
GSH	Reduced glutathione
H	hour
H_2O_2	Hydrogen peroxide
Kg	Kilogramme
MEq/L	Milliequivalent per litre
Mg	milligramme
min	minute
ml	milliltre
LSD	Least Significant Difference
NO	Nitric oxide
R_F	Retention factor
ROS	Reactive Oxygen Specie
SOD	Superoxide Dismutase

TCA	Tricarboxylic acid cycle
TLC	Thin Layer Chromatography

CHAPTER ONE

1.0 INTRODUCTION

For over a century, peptic ulcer disease has been one of the leading causes of gastrointestinal surgery, with high morbidity and mortality rates (Wang, 2000). The prevalence of gastrointestinal ulcers differs around the world; duodenal ulcers predominant in the western populations and gastric ulcers more frequent in Asia, especially in Japan (Hirano *et al*., 2001). In Africa, both duodenal and gastric ulcers affect the population (Yuan and Hunt, 2006). As the prevalence of this disease increases over time, peptic ulcers continue to have a significant global impact in the basic health and economic systems and in patient's life quality (Yuan and Hunt, 2006). It is estimated that approximately about 3000 people in the USA die as a result of the disease yearly with perforation being the most frequent indication for surgery. In Nigeria, its prevalence is estimated to be 10% of the population (Mustapha *et al*., 2007; WHO,2009).

Ulcer is often indicated by presence of symptoms such as epigastric pain, postprandial pain and nocturnal pain, and pain relieved by foods and antacids. Other complicated features include anemia caused by gastrointestinal blood loss, weight loss attributed to a reduced appetite, and vomiting (John, 2005). However, endoscopy is the most accurate diagnosis of gastric ulcer disease and its complications. These symptoms associated with gastric ulcers can result to inadequate food intake and consequently malnutrition (Wan, 1993). Risk factors for the development of gastric ulcers include excessive use of non steroidal anti-inflammatory drugs, *Helicobacter pylori* bacterial infection, stress, smoking, fatty foods, excessive intake of alcohol(Khazaei and Salehi, 2006). It is generally accepted that gastric ulcer results from an imbalance between aggressive factors (pepsin and acid) and protective factors (mucus, bicarbonate,

prostaglandins) in the stomach (Lima *et al.*, 2006; Jainu and Devi, 2006). It therefore results from over-bearing effect of stomach to these aggressive agents.

The suppression of acid secretions is a key therapeutic target for gastric ulcers, which includes the use of antacids, specific muscarinic receptor antagonists, targeting gastrin receptors and histamine receptors antagonists, and the use of proton pump inhibitors (Jain *et al*., 2007). These drugs are not completely effective and have some short comings such as high cost, relapse after treatment, drug interactions and other side effects such as hyperplasia, hypergastrophy and diarrhea in long term use (Khazaei and Salehi, 2006).

Natural products have been used for combating human diseases since they possess a wide range of compounds with significant biological activities that can be exploited for medical applications and other therapeutic purposes (Gupta *et al.*, 2012). Plants are recognized as important sources of natural compounds that protect from diseases and infections (Babincova *et al*., 2008). In developing countries, herbal preparations have played an important role side by side with modern medicine especially in the primary health care system of the poor and rural areas, and are increasingly utilized to treat a wide variety of clinical diseases including ulcer (Ezike *et al*., 2009). Many plants have been shown to possess anti-ulcerogenic property attributed to their flavonoids, tannins, alkaloids and phenolic contents (Alanko *et al*., 1999). Alanko *et al*. (1999) added that these metabolites are capable of scavenging reactive oxygen species affecting the arachidonate cascade via cyclo-oxygenase-2, have membrane stabilizing properties, increase mucosal content of prostaglandins and mucus in gastric mucosa; showing cytoprotective effects. They also prevent gastric lesions and protect the mucosa against necrotic agents (Alanko *et al*., 1999). In the process of wound healing, burns and inflammation, tannins help by forming protective layer of tannin-protein/ tannin-

carbohydrate complexes over the injured epithelial tissues, permitting the healing process to occur naturally (Simoes *et al.,* 2003). They also promote gastric mucosa formation, diminish acid secretion, inhibit production of pepsinogen and decrease ulcerogenic lesions (La Casa *et al*., 2000).

Ziziphus abyssinica belongs to the family Rhamnaceae that consists of small trees that are indigenous to tropical Africa and India. It is commonly called catch thorn in English and Magarya in Hausa (Nigeria). It is a spiny shrub up to 4m high that grows in the Sahel and drier part of tropical Africa. Its back is grey, deeply furrowed and branches are somehow zigzag with single or paired thorns of up to 12mm. Its cream pulp and outer skin are eaten. The pulp has a sweet to slightly bitter taste but edible portion is small (Chikamai *et al*., 2004). It has been used by Hausa people of northern Nigeria to treat abdominal ulcer and mental disorder. However, there is paucity of information on its efficacy. Thus, the present work is aimed at establishing the efficacy of *Ziziphus abyssinica* in the treatment of peptic ulcer and determine the constituents responsible for such action.

1.1 STATEMENT OF RESEARCH PROBLEM

Gastric ulcer is one of the most common chronic illness of the digestive system with symptoms such as upper abdominal pain, fullness, gas and bloating in some patients, while in others, it results to serious complications such as bleeding, bowel perforation, and obstruction. It is one of the most important causes of morbidity in both industrialized and non industrialized countries (Borrelli and Izzo, 2000). It is estimated that about 4 million people have gastric ulcers and approximately 3000 in the USA die

as a result of the disease yearly. In Nigeria, its prevalence is estimated to cause death of 5090 people in a year (WHO, 2009).

Several drugs have been employed for the treatment of gastroduodenal diseases and gastric ulcer resulting in a decrease in the mortality and morbidity rate. However, most of these drugs are not completely effective and have side effects (Rates, 2001). These adverse effects include high incidence of hip fractures and non-steroidal anti-inflammatory drugs-induced gastrophy (Rates, 2001), while long term use may result in diarrhea (Kakei *et. al.*, 1993), impotence, hypergastrinemia and hyperplasia (Howden and Hunt, 1994). Thus, there is a need for more effective and safer drugs with minimal or no side effects.

1.2 JUSTIFICATION

Ziziphus abyssinica is a plant used in treatment of peptic ulcers in the northern part of Nigeria. Studies by Nyaberi *et al.* (2010) have demonstrated the antibacterial and antioxidant properties of *Ziziphus abyssinica*. However, there is paucity of information on the efficacy and possible mechanism of antiulcer effect of the plant.

The rural population in various parts of the world does not have adequate access to high quality drugs for treatment of various ailments including ulcers, so they depend heavily on plants and herbal products. Thus, the great loss in terms of diagnosis and treatment and even depletion in human resources as a result of peptic ulcer diseases can be prevented with research to identify new therapeutic regimens that are non toxic, affordable and effective.

1.3 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to determine the gastro-protective effect of partially purified root extracts of *Ziziphus abyssinica* in ethanol induced ulcerated rats.

Specific objectives are to:

i. Investigate the gastro-protective effect of hexane, ethyl acetate, methanol and aqueous extracts of the roots of *Ziziphus abyssinica* in ethanol induced rats.
ii. Determine oral acute toxicity of the most active extract
iii. Fractionate the most active crude extract using solvent partitioning and determine gastro-protective effect of the fractions.
iv. Investigate the *in vivo* antioxidant potential of the fractions.
v. Partially purify the most active fraction using column chromatography and determine the antiulcer effect of the fractions.

1.4 Hypothesis

The extracts and fractions of *Ziziphus abyssinica* root do not protect from ethanol induced ulcer in rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Medicinal Plants

The use of natural products, especially plants, for healing is as ancient and universal as medicine itself. The therapeutic use of plants certainly goes back to the Sumerian civilization, and 400 years before the Common Era, it has been recorded that Hippocrates used approximately 400 different plant species for medicinal purposes. Natural products played a prominent role in ancient traditional medicine systems, such as Chinese, Ayurveda, and Egyptian, which are still in common use today. According to Gupta *et al.* (2012), 75% of people still rely on plant-based traditional medicines for primary health care globally. A brief summary of the history of natural product medicine is presented in Table 2.1.

Table 2.1: *History of natural product medicine*

Period	Type	Description
Before 3000 BC	Ayurveda	Introduce medicinal properties of plants and other natural Products
1550 BC	Ebers Papyrus	Presented a large number of crude drugs from natural sources (e.g castor seeds and gum Arabic)
460-377 BC	Hippocrates, "The Father of Medicine"	Described several plants and animals that could be sources of medicine
370-287 BC	Theophrastus	Described several plants and animals that could be sources of medicine
23-79 AD	Pliny the Elder	Described several plants and animals that could be sources of medicine
60-80 AD	Dioscorides	Wrote *De Materia Medica*, which described more than 600 medicinal plants
131-200 AD	Galen	Practiced botanical medicine (Galenicals) and made them popular in the West
15th century	Krauterbuch	Presented information and pictures of medicinal plants

(Cragg *et al*., 1997).

2.1.1. Significance of *Ziziphus abyssinica* in Ethnic Folk Medicine

Ziziphus abyssinica is both a delicious fruit and an effective herbal remedy. It aids weight gain, improves muscular strength and increases stamina. In Chinese medicine, it is prescribed as a tonic to strengthen liver function. It functions as antidote, diuretic, emollient and expectorant. The leaves are astringent and febrifuge and also said to promote hair growth. The dried fruits are anodyne, anticancer, pectoral, refrigerant, sedative, stomachache, styptic and tonic. It is considered to purify blood and aid digestion. It is used internally in the treatment of chronic fatigue, loss of appetite, diarrhea, anemia, irritability and hysteria. The plant is a folk remedy for anemia, hypertonia, nephritis and nervous diseases. The fruits are applied on cuts and ulcers and also employed in pulmonary ailments and fevers. The leaves are applied as poultices and are helpful in liver troubles, asthma and fever (Devi *et al*., 1989; Azam-Ali *et al.,* 2012).

2.1.2. Morphology and Geographical distribution of *Ziziphus abyssinica*

Ziziphus abyssinica is a spiny shrub up to 5m high, with trunk 20cm or more in diameter; spreading crown; stipular spines and many drooping branches. The fruit is variable in size and shape. It is oval, obovate, oblong or round, and can be 2.5-6.25 cm long, depending on the variety. The fruits skin is smooth, glossy, thin and tight. It is most commonly found in the tropical and sub-tropical regions. It is found in India, Africa, Afghanistan, China, Malaysia and Australia. It is fast growing with a medium life span that can reach up to 10-40 ft tall. (Kundu *et al*., 1989; Pawlowska *et al.,* 2000; Sarfaraz *et al.,* 2002; Shiv *et al*., 2004).

2.1.3. Scientific classification of *Ziziphus abyssinica*

Kingdom	Plantae
Division	Magnoliophyta
Subdivision	Angiosperm
Class	Magnoliopsida
Order	Rosales
Family	Rhamnaceae
Genus	*Ziziphus*
Species	*Abyssinica*

Binomial Nomenclature *Ziziphus abyssinica* (Kanitkar, 1969).

2.1.4 Biological effects of *Ziziphus abyssinica*

Studies by Nyaberi *et al.* (2010) on evaluation of phytochemicals in fruit extract of *Z. abyssinica* revealed the presence of alkaloids, flavonoids, tannins, saponins, triterpenes, steroids and sterols. The study also revealed the antibacterial effect of the plant on *Staphylococcus aureus, Pseudomonas aeruginosa, Esherichia coli and Candida albicans*. The study explained the reason for its use as preservative to preserve meat in Kenya. Studies by Ugwa *et al.* (2011) have shown that this plant is an effective remedy for diarrhea.



Plate I: Picture of *Ziziphus abyssinica* by Salahuddeen Yau on 28 September 2014 along Nasarawa road in Faskari.

2.2.0 **Overview of Ulcer Patho-physiology**

Peptic ulcer is one of the world's major gastro-intestinal diseases, embracing both gastric and duodenal ulcers, and affecting 10% of the world population. For decades, it was believed that gastrointestinal ulcerations were caused by the excessive secretion of gastric acid, but many patients presenting such ulcerations had normal acid secretion rates (Tarnawski, 2005). Then researchers reported that peptic ulcers were been caused by an imbalance between the aggressive factors and a number of known defense mechanisms. Exogenous aggressive factors such as smoke, anti-inflammatory drugs, alcohol, stress, fatty foods and *Helicobacter pylori* infections triggered tissue necrosis through mucosal ischemia, free radical generation and cessation of nutrient delivery, hydrochloric acid together with pepsin, pancreatic enzymes and bile decreased the defense mechanisms of gastrointestinal mucosa such as intercellular junctions, local blood flow, mucus/bicarbonate secretion and cellular growth (Bandyopadhyay *et al*., 2001). These aggressive factors produce pathological changes and the development of inflammation, hemorrhagic erosions, and ulcers with the acute involvement of free radicals or Reactive Oxygen Species (Wada *et al*., 1997; Kwieciente *et al.*, 2002; Odabasoglu *et al.,* 2006).These radicals are normally neutralized by the action of antioxidant defense system consisting of organic substances containing thiol groups such as glutathione, vitamins C and E, NADPH, antioxidant enzymes such as peroxidase, glutathione reductase and others (Chattophadhyay *et al*., 2006). When there is imbalance between ROS and antioxidant defense mechanisms, ROS lead to oxidative modifications in the cellular membrane and intracellular molecules resulting in peroxidation of membrane lipids, accumulation of lipid peroxides, and cellular damage (Cartea *et al*., 2011).

2.2.1 **Causes of Peptic Ulcer**

2.2.1.1. **Non Steroidal Anti-inflammatory Drugs (NSAIDs)**

NSAIDs play a critical role in development of gastric ulcer by inhibition of cycloxygenase activity, an enzyme responsible for the synthesis of prostaglandins from arachidonic acid thereby leading to decreased mucosal prostaglandins. Prostaglandins were believed to accelerate ulcer healing by inhibiting gastric acid secretion, stimulate endothelial cells to release more bicarbonate and mucus, and reduce the permeability of epithelium (Takeuchi *et al.,* 1994). Aspirin has been found to inhibit cycloxygenase irreversibly, while other NSAIDs inhibit cycloxygenase in a reversible, concentration dependent manner (Cryer and Spechler, 2006). There are two isoforms of cycloxygenase (COX); cycloxygenase-1 and cycloxygenase-2. Cycloxygenase-1 is constitutively expressed in most tissues including stomach and is responsible for maintaining gastric mucosal integrity whereas cycloxygenase-2 participates in inflammation (Cryer and Spechler, 2006). Studies by Laine *et al*. (2008) demonstrated that both COX-1 and COX-2 may play a role in prostaglandin synthesis and maintenance of gastric mucosal integrity, and that COX-2 plays a back-up role by alleviating prostaglandin deficiency induced by COX-1.

Other mechanism proposed to explain NSAID-induced stomach injury is "trapping theory" in that NSAID are weak organic acids. In gastric juice, they are non-ionized and lipid-soluble. These NSAIDs diffuse across gastric mucosal epithelial cell membrane into the cytoplasm, where the pH is neutral. At neutral pH, NSAIDs are converted into the reionized and relatively lipophilic form, and are therefore trapped and accumulate within cells leading to cellular injury (Laine *et al.,* 2008).

The third mechanism is its effect on mitochondrion; an organelle responsible for energy generation and regulation of cell death (Ott *et al.,* 2007). The respiratory chain of mitochondria is the major source of reactive oxygen species (ROS) generated mainly at complex I and complex III of the respiratory chain. These ROS generated from mitochondria play a role in the release of cytochrome c and other pro-apoptotic agents which trigger caspase activation and apoptosis Ott *et al.* (2007).

Mitochondria are the main target of absorbed NSAIDs. NSAIDs inhibit, uncouple oxidative phosphorylation to dissipate mitochondria transmembrane potential, leading to liberation of cytochrome c into the cytosol out of mitochondrial intermembrane space and to the release of ROS such as hydrogen peroxide, superoxide (O_2^-) thereby causing activation of caspase 9 and caspase 3 and therefore cellular lipid peroxidation resulting in apoptosis(Brand *et al.*,2004; Nagano *et al*., 2005). The uncoupling of mitochondria decreases the ATP concentration, leakage of Ca^{2+} out of mitochondrion, cellular osmotic imbalance and loss of control over cellular junctions, resulting in increased permeability and subsequent mucosal damages (Somasundarum *et al.*, 1995; Maity *et al*., 2008)

2.2.1.2 **Stress**

Stress has been proven to be involved in the etiology and patho-physiology of peptic ulcer through several mechanisms.

Oxidative stress results from imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses of the body, with potential to cause oxidative deterioration of proteins, lipids, and DNA (Halliwell, 1991). ROS generation is

elevated by different stress conditions, and these can attack vital cell components like Polyunsaturated Fatty acids, proteins and nucleic acids. These reactions alter the membrane fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage; ultimately resulting in cell death (Hiruma-Lima *et al.*, 2006).

Under stress conditions, nitric oxide produced by inducible nitric oxide synthase reacts with superoxide anion produced by neutrophiles forming peroxynitrites. These nitric oxide derived reactive compounds contribute to the nitrosative and oxidative stress burden of mucosal layer of the stomach and duodenum (Kobata *et al.* 2007).Cold-restraint stress induces myeloperoxidase activity significantly by activation of neutrophiles. These activated neutrophiles produce many enzymes and free radicals that damage gastric mucosa (Nishida *et al.*, 1997).

Studies have shown that stress causes elevation or increased levels of cathecolamine associated with vasoconstriction of blood vessels, consequently causing hypoxia and reduced supply of nutrients to the mucosal epithelial cells required for the synthesis of prostaglandins, mucus and other endothelial growth factors for cytoprotection and therefore disruption of gastric motility (Safwan *et al.*, 2011). Vasoconstriction and gastric hypermotility can impair gastric microcirculation by decreasing blood supply to the mucosal cells, decreased prostaglandin synthesis and increased formation of gastric lesions.

2.2.1.3 Mechanism of ulcer induction by ethanol

Ethanol is an organic compound containing two carbon atoms and hydroxyl functional group. Exposure to ethanol induces gastric damage due to its direct action on gastric mucosal epithelium causing lipid peroxidation, oxidative stress and mitochondrial

permeability transition and mitochondrial depolarization which lead to cell death, decreased nitric oxide release, increased endothelial nitric oxide synthase protein expression in response to the nitric oxide demand (Bandyopadhyay *et al*., 2001).

In other words, ethanol induces damage to the gastric mucosa by activation of phagocytes to produce superoxide anion, hydrogen peroxide, nitric oxide, liberation of arachidonic acid, and peroxide enzymes to produce alcohoxyl and peroxyl radicals which attack lipids and proteins (Bandyopadhyay *et al.,* 2001). It is also suggested that ethanol leads to liberation of ions of transition metals which stimulate a change in H_2O_2 to HO; conversion of xanthine dehydrogenase to xanthine oxidase from damaged cells to produce systemic damage, and increase hypoxanthine levels by rupture of energy metabolism. The mitochondrial damage produces an increase of electron transfer which, in turn produces superoxide anion and increase intracellular Ca^{2+} levels, that trigger the release of nuclease activity and Ca^{2+} dependent nitric oxide synthase, generation of more NO from ONOO leading to oxidative stress (Suzuki *et al*., 1994).

Another hypothesis proposed to explain the ethanol-induced oxidative damage to the gastric mucosa is the constrictive effect of ethanol on veins and arteries of the gastric mucosa thereby producing congestion, inflammation, and tissue injury. It also increases gastric motility and decreases mucus secretion (Bandyophadhyay *et al*., 2001).

2.2.1.4 ***Helicobacter pylori***

Helicobacter pylori is a gram negative, microaerophilic bacterium, 3 micrometers long with diameter of 0.5 micrometers that colonizes the human stomach. It contains hydrogenase used to obtain energy by oxidizing molecular hydrogen and produces

oxidase, catalase, urease. It is capable of forming biofilms, and can convert from spiral to a visible non culturable coccoid form that favor its survival and as factors in the epidemiology of the bacterium.

Helicobacter pylori are risk factor for the development of peptic ulcer and gastric adenocarcinoma (Suerbaum and Michetti, 2005). The vacuolating cytotoxin (Vac A) is a major virulence factor that causes cell vacuolation and subsequent tissue damage (Tombolo *et al*., 2003).

In the stomach, it avoid the acidic environment of the interior stomach by using flagella to burrow in to the mucus lining the stomach to reach the epithelial cells underneath where there is a more neutral pH. *Helicobacter pylori* are also able to sense the pH gradient in the mucus and move towards the less acidic region. This keeps the bacteria from being swept away into the lumen with bacteria's mucus environment which constantly moves from site of production at the epithelium to the lumen interface where it produces adhesions (Tombolo *et al.,* 2003).

Reactive Oxygen Species (ROS) released from activated neutrophiles are also potential virulence factors involved in *H. pylori*-infected host cells. In *H. pylori*- infected host cells, hypochlorous anions are generated from H_2O_2 in the presence of Cl^-. The hypochlorous anions subsequently react with ammonia, which is derived from urea by urease produced by *H. pylori*, ultimately yielding monochloramine (NH_2Cl). NH_2Cl induces mucosal cytotoxicity due to its lipophilic properties and freely penetrates biological membranes to oxidize intracellular components (Suzuki *et al*. 1994).

In addition, it is well-known that *H. pylori* produces a gamma-glutamyltranspeptidase (GGT) in the periplasm (Suzuki *et al.,* 1994). *H. pylori* GGT catalyzes the transpeptidation and hydrolysis of the glutamyl groups of glutamine and glutathione.

These findings indicate that *H.pylori* is unable to take up extracellular glutamine and glutathione directly; GGT hydrolyzes these substances to glutamate. The glutamate is then transported into *H. pylori* cells via a Na^+-dependent reaction and is mainly incorporated into the TCA cycle.

Excess Reactive Oxygen Species are produced in *H. pylori*-colonized human stomach, this induces oxidative stress to both the gastric mucosa and *H. pylori*. Because *H. pylori* has a deft capability of detoxifying ROS using a variety of enzymes to establish long-term colonization, excess ROS leads solely to host cell damage (Seyler *et al*., 2001).

2.2.2 **Cytoprotective Factors for Ulcer**

2.2.2.1 **Gastric Mucus**

Gastric mucus is an important protective factor for the gastric mucosa. It is a viscous, elastic, adherent, transparent gel formed by 95% water and 5% glycoproteins, that covers the entire gastrointestinal mucosa. It creates an unstirred layer on the mucosal surface which supports a near neutral pH at surface and a physical barrier against pepsin (Aniagu *et al*., 2005).

Decrease in gastric mucus render the mucosa susceptible to injuries induced by acid, aspirin and cold-restraint stress. Moreover, mucus is capable of acting as antioxidant, and thus can reduce the mucosal damage mediated by oxygen radicals. The protective properties of the mucus barrier depend not only on the amount or thickness of the layer covering the mucosal surface (Penissi and Piezzi, 1999). It is suggested that if some oxygen radicals are produced in surface epithelium containing mucus, intracellular mucus may be released into the gastric tissue and prevent additional damage by

scavenging them. The mucus membrane of gastrointestinal tract contains a rich amount of mucin; a protein involved in mucosal protection (Longman *et al.,* 2000). The luminal surface mucin act as protective barrier. The high molecular weight mucins are responsible for the viscoelastic properties of mucus barrier. The major mucins found in gastrointestinal tract are mucin 2 and mucin 6 which form covalent polymers and have bactericidal action, so protect against ulcer (Vasconcelos, 2010).

2.2.2.2 **Prostaglandins**

Prostaglandins are hormone-like lipids derived enzymatically from fatty acids, with 20 carbon atoms and a 5-carbon ring. They are found in most tissues and organs, and are produced by all nucleated cells. They are autocrine and paracrine lipid mediators acting on platelets, endothelium, uterine and mast cells (Nelson, 2005). Prostaglandins are produced following sequential oxidation of arachidonic acid by cycloxygenases (COX-1 and COX-2) and terminal prostaglandin synthases. Cycloxygenase-1 is responsible for the baseline levels of prostaglandins while cycloxygenase-2 produces prostaglandins through stimulation. However, while COX-1 and COX-2 are both located in the blood vessels, stomach and kidneys, prostaglandin levels are increased by COX-2 in scenarios of inflammation.

Prostaglandins have been shown to increase the amount of luminal mucus and the resistance of the gastrointestinal tract to injury in many experimental models. They exert their cytoprotective effect through the stimulation of mucus and bicarbonate secretion, maintenance of mucosal blood flow, and enhancing the resistance of epithelial cells to injury induced by cytotoxins (Nelson, 2005).

2.2.2.3 **Antioxidants**

Antioxidants are substances and or nutrients in our foods which prevent or delay oxidative damage to our body. They are classified as either enzymatic (endogenous) antioxidants or non-enzymatic (exogenous) antioxidants. Under normal conditions, antioxidants convert ROS to H_2O to prevent overproduction of reactive oxygen species (La Casa *et al.,* 2000).

Enzymatic Antioxidant

These are also known as natural antioxidants. They neutralize Reactive Oxygen Species and prevent it from damaging the cellular structure and include superoxide dismutase (SOD), Catalase and glutathione peroxidases.

Superoxide Dismutase

Superoxide dismutase is a metalloprotein found in both prokaryotic and eukaryotic cells (Haraguchi *et al*., 2000). The iron-containing and manganese containing enzymes are characteristic of prokaryotes. In eukaryotes, the predominant forms of the enzyme are copper-containing enzyme and zinc-containing enzymes located in the cytosol. Manganese containing enzyme is found in the mitochondrial matrix. Superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Thus catalyzes the destruction of oxygen free radical

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

Glutathione Peroxidase

Glutathione peroxidases catalyzes the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction product of hydroperoxides

(Yelken, *et al*., 1999). Glutathione consists of three amino acids, (glutamate, cysteine and glycine) is an essential component of this system and a cofactor for the enzyme glutathione transferase.

$H_2O_2 + GSH \longrightarrow GSSG + H_2O$

$GSSG + NADPH \longrightarrow GSH + NADP^+$

Catalase

Catalase is a heme –containing enzyme that catalyzes the destruction of hydrogen peroxide into water and oxygen. The enzyme is found in eukaryotes, and is important in the removal of hydrogen peroxide generated in peroxisomes by oxidases involved in beta oxidation of fatty acids, glyoxylate cycle and purine catabolism (Yelken *et al.*, 1999).

$2H_2O_2 \longrightarrow 2H_2O + O_2$

2.3 **Drugs Used in the Management of Ulcer**

Several therapeutic interventions have help in the treatment and prevention of ulcer. Conventional medications used in the treatment of ulcer include antacids, broad spectrum antibiotics, histamine (H_2) receptor antagonist, proton pump inhibitors, cytoprotective drugs such as mistoprostol and sucralfate.

2.3.1 **Histamine Receptor Antagonist**

Histamine is an organic nitrogenous compound derived from amino acid histidine in a reaction catalyzed by decarboxylase. It is produced by basophils and mast cells and involved in local immune responses and inflammatory response (Marieb, 2001).

Histamine exerts its effects by binding to G-protein coupled histamine receptors, H_1 through H_4. In binding to the H_2 receptor, histamine is protonated at the end-chain amine group which interacts with aspartic acid in the transmembrane domains of the receptor. The other nitrogens interact with threonine and aspartic acid of transmembrane domains bringing the domains close to each other which initiates signal transduction cascade (Yanai and Tashiro, 2007).

During gastric acid release in the stomach, enterochromaffin-like cells located in the gastric glands of stomach release histamine that stimulates parietal cells by binding to apical H_2 receptors. Stimulation of parietal cells induces the uptake of carbon dioxide and water from the blood which is then converted to carbonic acid by carbonic anhydrase. In the cytoplasm of the parietal cells, the carbonic acid readily dissociates in to hydrogen and bicarbonate ions. The bicarbonate ions diffuse through the basilar membrane into the blood stream, while the hydrogen ions are pumped into the lumen of the stomach by H^+/K^+ ATPase pump (Alvarez, 2007).

Histamine 2 receptor antagonists are widely used in the treatment of acid-peptic diseases. The H2 rceeptor blockers act by binding to the H2 receptors on the basolateral surface of gastric parietal cells, interfering with pathways of gastric acid production and secretion (Alvarez, 2007). They are less potent in inhibiting acid production than proton pump inhibitors but suppress 24 hour gastric acid secretion by 70%. Examples of H2 receptor antagonists are cimetidine, ranitidine, famotidine and nizatidine. Reported side effects of H2 rceptor antagonists include diarrhea, constipation, fatigue, drowsiness, headache and muscle pain in some patients (Alvarez, 2007).

2.3.2 **Proton Pump Inhibitors**

These are inhibitors/ antisecretory agents an example is omeprazole; a weak basic drug with pka 3.97 that acts by irreversibly blocking the H^+/K^+ ATPase (proton pump), the terminal step in the acid secretory pathway. This drug is inactive at neutral pH, but accumulates in an acid environment and is activated at a pH lower than 3 by protonation (John, 2005). Omeprazole is thought to react with sulphydryl groups associated with the proton pump and markedly inhibits both basal and stimulated gastric secretion. The preferential accumulation of the drug in areas of gastric parietal cells means that it has a specific effect on these cells (Rang, 2003). Side effects are minor and include hyperplasia and hypergastrinemia leading to the formation of carcinoid tumor and an increase in bacteria in the stomach which, in time, could lead to an increase in nitrates and carcinogenic N-nitrosamines.

2.3.3 **Muscarinic Receptor Antagonist**

The main effects of parasympathetic stimulation on the gastrointestinal tract are an increase in gastric motility and increased in secretory activity. Anticholinergics are used to abolish gastric secretion provoked by parasympathetic agents but have a partial effect on histamine-induced secretion and decrease gastric motility (Rang, 2003). An example is pirenzepine; a strongly hydrophilic drug, with poor penetration in to the central nervous system, and binds effectively to the sub-class of muscarinic cholinergic receptors of the gastric mucosa, resulting in effective inhibition of acid and pepsin secretion without significant anticholinergic effects elsewhere. Reported side effects include blurred vision, photophobia, constipation, urine retention, oxcanal impotence (John, 2005).

2.3.4 Antacids

These are acid opposers which act by neutralizing hyperchloridia and therefore raise the gastric pH, which has the effect of inhibiting peptic activity and produce prompt gastric relief in patients. They are classified as either of systemic effect or non systemic effect. The systemic antacids alter the pH of the extracellular fluid, and are rapid in action but usually produces carbon dioxide. An example is sodium bicarbonate. Those without systemic effect have a slow action due to their insolubility and may produce diarrhoea and their aluminium salts are constipating. Examples are magnesium trisilicates, oxides and hydroxides (John, 2005).

2.4 **Some Plant Secondary Metabolites That Protect From Ulcer**.

2.4.1 **Flavonoids**

Flavonoids are diphenylpropanes that commonly occur in plants (more than 4000 flavonoids have been found) and are frequently components of human diet. Family members include flavones, isoflavones, 2, 3-dihydroderivatives of flavonoids (flavonones). These flavonoids with multiple OH substitution have strong antioxidant activities against peroxyl radicals (Alanko *et al.*, 1999).

The principal properties that account for the potential health benefits of flavonoids is their antioxidant activity. Several in vitro antioxidant studies have demonstrated that flavonoids can scavenge superoxide, hydroxyl and peroxyl radicals that affect various steps in the arachidonate cascade through cyclooxygenase-2 or lipoxygenase (Alanko *et al.*, 1999). They also increase the stability of membrane, and affect some processes of intermediary metabolism and inhibit lipid peroxidation in different system.

Some flavonoids have been shown to increase the mucosal contents of prostaglandins and mucus in gastric mucosa, showing cytoprotective effects. Several of these flavonoids prevent gastric mucosal lesions produced by various experimental models and protect gastric mucosa against necrotic agents (Alanko *et al*., 1999).

Several mechanisms account for the antioxidant activity of flavonoids in addition to radical scavenging activities such as chelating of transition metals ions, inhibition of oxidant enzymes, production of free radicals by cells, and regeneration of tocopherol radicals. They also promote gastric mucosa formation, diminish acid secretion, inhibit production of pepsinogen and decrease ulcerogenic lesions (Bansal and Goel, 2012).

Flavonoids may be regarded as active compounds against gastric lesions by acting as protective factors or increasing antioxidant activity. The mechanism may be by alterations of glutathione metabolism, quenching of reactive oxygen species and the inhibition of Ca^{2+} influx that signals the last step in the cell death cascade induced by glutamate (Ishige *et al*., 2001).

2.4.2 **Tannins**

Tannins are polyphenols found in plants, foods and beverages, water soluble with molecular weight from between 500 and 3000 daltons, and of great economic and ecological interest, that form complexes with water-soluble proteins, alkaloids and gelatin. They are responsible for the astringent taste of many fruits and vegetables, causing precipitation of salivary glycoproteins and reducing oral lubrication (Albuquerque *et al*., 2005).

Being phenolic compounds, tannins are chemically reactive and form inter and intra-molecular hydrogen bonds. They are easily oxidized by specific plant enzymes and

influenced by metals such as ferric chloride (which causes darkening solution). Classically, the chemical structures of tannins are divided into two groups: hydrolysable and condensed. The hydrolysable tannins consist of gallic acid esters, and ellagic acid glycosides, formed from shikimate where the hydroxyl sugars are esterified with phenolic acids.

Many plant species producing tannins are used in folk medicine for different purposes. They are used in drug applications due to their astringent properties. They exert internal and antidiarrheal and antiseptic effects by water proofing the outer layer of more exposed mucous membranes. Precipitating proteins, tannins provide antimicrobial and antifungal effects. In the process of wound healing, burns and inflammations, tannins help by forming protective layer (tanning-protein complex/ tannin-carbohydrate complex), over the injured epithelial tissues permitting the healing process below to occur naturally (Simoes *et al*., 2003).

Tannins react with proteins of the tissue layer. Tannins precipitate microproteins at the site of the peptic ulcer, forming a protective pellicle that prevents absorption of toxic substances and promote resistance to the action of proteolytic enzymes, and an associated activity against *Helicobacter pylori* (Vasconcelos *et al.,* 2008). In several experimental models of gastric ulcer, purified tannins have been shown to be involved with gastrointestinal tract anti-inflammatory actions, promotion of tissue repair, acid secretion inhibition, and both antioxidant and anti*Helicobacter pylori* activity.

Many phenolic compounds including catecholamines have been shown to modulate prostaglandins synthase and 5-lipoxygenase pathways of arachidonic acid (Alanko *et al.,* 1999). Phenols have two effects on prostaglandin biosynthesis, with low concentrations stimulating the synthesis of prostaglandins and high concentrations

inhibiting prostaglandin synthase. They stimulate the synthesis by acting as reducing substrates for the oxidized intermediates of prostaglandin synthase, thereby accelerating the peroxidase cycle and by functioning as electron- donating cosubstrates for the peroxidase components of prostaglandin synthase. Phenols inhibit cyclooxygenase-2 activity of prostaglandins by competing for the arachidonic acid-binding site and by competitive reduction of prostaglandins. The modulation of hydroperoxide tone by phenols is probably the key element explaining suppression of arachidonic acid metabolism by prostaglandins. The stimulatory effect of phenols on prostaglandin E_2 formation may be based on their action as co-substrates for the peroxidase reaction Alanko *et al.* (1999).

2.4.3. **Alkaloids**

Alkaloids are a group of natural products with diverse group of low molecular weight nitrogen-containing compounds derived mostly from amino acids (Ziegler *et al*., 2008). These secondary metabolites are found in 20% of plant species and are classified as true alkaloids, which have the nitrogen atoms in heterocyclic rings and pseudoalkaloids, which are not derive from amino acids but may have nitrogen atoms in heterocyclic rings (Henriques *et al*., 2004).

Several alkaloids are being utilized in therapeutic and as pharmacological tools. The reported biological activities are anticholinergics, antitumor, diuretic, sympathomimetic, antimicrobial, antiviral, antihypertensive, antidepressant, hypoanalgesic, emetic (Henriques *et al*., 2004). Studies have shown that alkaloids are useful pharmacological tools to discover new active principles of gastric mucosa against the damaging effects induced in rats by indomethacin, reserpine, stress, pylorus ligature, absolute ethanol (Wang, 2000).

Several studies revealed the gastroprotective and ulcer healing activity of alkaloids by inhibition of acid secretion and gastric motility, stimulation of alkali/mucus secretion, increase mucosal blood flow which all help in prevention and healing of ulcers against injury caused by aggressive agents. Piperidine alkaloid was found to protect gastric mucosal damage caused by stress, indomethacin, ethanol, pylorus ligation by decreasing volume of gastric juice, gastric acidity and pepsin activity (Bai and Xu, 2000). In the stomach, capsaicin alkaloid have been found to protect stomach by inhibiting acid secretion, stimulating the alkali/mucus secretions and increase the gastric mucosal blood flow which help in prevention and healing of ulcers against injury caused by aggressive agents (Konturek *et al.*, 2006).

Moreover, alkaloids of the indole class have been found to posses antiulcer activity or protect the gastric mucosa from the damage caused by ischemia-reperfusion and absolute ethanol through attenuation of the gastric blood flow failed and scavenging of free radicals Konturek *et al.* (2006).The pyrrolidine alkaloids were also found to demonstrate significant activity in acute and chronic gastric ulcers. These alkaloids increased free mucus and prostaglandins in the gastric mucosa. Moreover, they showed a reduction of exfoliation of superficial cells, hemorrhages and blood cell infiltration mediated by increase in gastrin secretion and mRNA expression of epidermal growth factors (Toma *et al.*, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals/ Reagents

All the chemicals used were of analytical grade and include: Absolute ethanol, chloroform, ethyl acetate (BDH laboratories); Methanol, butanol, hexane (Guangdong Guanghua Scientific Company Ltd China); Cimetidine (Brussels laboratories Pvt. Ltd India). All other chemicals and reagents used were also of analytical grade.

3.1.2 Experimental animals

One hundred and twenty two (122) adult albino rats (Wistar strain) of both sexes weighing between 150-200g were obtained from animal house, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were fed normal rat chow and allowed access to clean water *ad libitum.* The rats were kept in wire mesh cages for a period of two weeks to acclimatize before the commencement of the experiment.

3.1.3 Plant Material Collection

The roots of *Ziziphus abyssinica* were collected from Faskari, Faskari Local Government Area of Katsina State, Nigeria. The plant was identified and authenticated at the herbarium unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State. The voucher number of the plant is 257.The roots were sliced into small sizes, dried under the shade, pulverized into moderately coarse powder using pestle and mortar, then stored in sealed plastic containers until required.

3.2 METHODS

3.2.1 **Preparation of the extracts**

Exactly 300g each of the powdered root sample of *Ziziphus abyssinica* was soaked in 0.9 litre hexane, ethyl acetate, methanol and distilled water for 48 h and then sieved using muslin cloth to obtain hexane, ethyl acetate, methanol and aqueous extracts. Then the resulting extracts were filtered with Whatman No. 1 filter paper. All extracts were concentrated using water bath maintained at a temperature of 40-45^{0}C and stored at 4^{0}C until required.

3.2.2 **Anti-ulcerogenic Study of the extracts: Grouping**

Fifty five (55) albino rats were randomly divided into eleven groups of five rats each.

Group1: Rats were given food and water. This group serves as normal control group.

Group2: Rats were given food and water (not pretreated with the extract); fasted 24 hours and 1ml of 95% ethanol administered by oral gavage. This group serves as ulcer control group.

Group 3: Rats were pretreated with hexane (300mg/kg b.w.) for fourteen days; fasted for 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

Group 4: Rats were pretreated with hexane extract (600mg/kg b.w.) for fourteen days; fasted 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

Group 5: Rats were pretreated with ethyl acetate extract (300mg/kg b.w.) for fourteen days; fasted 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

Group 6: Rats were pretreated with ethyl acetate extract (600mg/kg b.w.) for fourteen days; fasted 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

Group 7: Rats were pretreated with methanol extract (300mg/kg b.w.) for fourteen days; fasted 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

Group 8: Rats were pretreated with methanol extract (600mg/kg b.w.) for fourteen days; fasted 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

Group 9: Rats were pretreated with aqueous extract (300mg/kg b.w.) for fourteen days; fasted 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

Group 10: Rats were pretreated with aqueous extract (600mg/kg b.w.) for fourteen days; fasted 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

Group 11: Rats were pretreated with cimetidine (100mg/kg b.w.) for fourteen days; fasted 24 hours after the last treatment and 1ml of 95% ethanol administered orally.

3.2.3. **Gastric Ulcer Induction**

One hour after administration of ethanol, rats were sacrificed with clean razor blade under chloroform anesthesia. The gastric portion of each rat was removed and washed in physiological saline solution. Ulcers were assessed and observed using the modified method of Magistretti *et al.* (1988).

3.2.4**. Ulcer Index**

Gastric lesions in the glandular region of the stomach were located in the gastric mucosa and determined by the number and severity of lesions as described by the modified Magistretti *et al.* (1988) method as follows:

0=No lesion, 0.5= Hemorrhage, 1=1 to 3 small lesions < 1mm length, 2= 1 to 3 large lesions > 1mm length, 3= 1thickened lesions, 4= more than 3 small lesions 5= more than 3 large lesions, 6= more than 3 thickened lesions.

This method was also used for fractions obtained from solvent partitioning and fractions from column chromatography.

3.2.5. **Percentage Inhibition (PI)**

This was calculated according to the method of (Hano *et al*., 1976) as follows:

$$PI = \frac{\text{mean ulcer index (control) - mean ulcer index (treated group)}}{\text{Mean ulcer index (control).}} \times 100$$

The same method was used for fractions from solvent partitioning and fractions from column chromatography.

3.2.6 **Median Lethal determination**

Median lethal dose of the most active extract of the roots of *Ziziphus abyssinica* was carried out with the method of Organization for Economic and Cooperative Development (OECD, 2001). In this method, five groups of one rat each were administered 3000mg/kg body weight of the most active extract (aqueous extract) one after the other. Observation for toxic symptoms was made and recorded at 1, 2, 4 and 6 hr after administration. Finally, the number of survivors was noted after 48 hr for each animal. The toxicological effect was assessed using the method of OECD (2001) on the basis of mortality, limit test dose up and down procedure.

3.2.7. **Fractionation of the most active extract**

Exactly 60.3g of aqueous extract was fractionated with 1.2 litres of hexane, ethyl acetate and butanol in a separating funnel. Four fractions were obtained; hexane, ethyl acetate, butanol and remaining aqueous fraction. The yields were 0 for hexane; 0.76g for ethyl acetate fraction; 21.3g for butanol fractions and 37.4g for aqueous fractions.

3.2.8 **Antiulcerogenic study of butanol and aqueous fractions: Grouping**

Thirty five (35) adult albino rats were randomly divided into seven (7) groups of five animals each. The rats were grouped as follows:

Group1: Rats were given food and water (not pretreated with either fraction) and ethanol not administered. This group serves as the normal control group.

Group 2: Rats were given food and water, fasted 24 hour and 1ml of 95% ethanol administered orally. This group serves as ulcer control group.

Group 3: Rats were pretreated with cimetidine (100mg/kg b.w.) for ten days, fasted 24 hour and 1ml of 95% ethanol administered orally. This group served as standard control group.

Group 4: Rats were pretreated with butanol fraction (100mg/kg b.w.) for ten days, fasted 24 hour and 1ml of 95% ethanol administered orally.

Group 5: Rats were pretreated with butanol fraction (200mg/kg b.w.) for ten days, fasted 24 hour and 1ml 0f 95% ethanol administered orally.

Group 6: Rats were pretreated with aqueous fraction (100mg/kg b.w.) for ten days, fasted 24 hour and 1ml of 95% ethanol administered orally.

Group 7: Rats were pretreated with aqueous fraction (200mg/kg b.w.) for ten days, fasted 24 hour and 1ml of 95% ethanol administered orally.

3.2.8.1 **Collection of Sample**

One hour after ethanol was administered, rats were anesthetized with chloroform anesthesia and sacrificed by cervical dislocation using clean razor blade. Stomach of each rat was open and excised, contents collected into test tubes. The gastric tissue was harvested for further studies (Kikuko *et al*., 1999).

3.2.**9 Determination of Gastric Ulcer Parameters**

3.2.9.1. **Determination of gastric juice volume**

Gastric juice was centrifuged at 4000 rpm for 10 mins. The resulting supernatant was collected and volume determined and expressed in milliliter (Kikuko *et al*., 1999). The same procedure was used to fractions obtained from column chromatography.

3.2.9.2. **Determination of gastric juice pH**

One milliliter of the supernatant liquid was pipetted out from each test tube. The pH of the supernatant was determined with the aid of a digital pH meter. The same method was used to fractions from column chromatography.

3.3.0 **Biochemical Analysis**

3.3.1**. Estimation of total and free acidity**

One milliliter (1ml) of gastric juice sample was pipetted into a 250ml conical flask and 2 drops of methyl orange was added and titrated against 0.01M NaOH till the red color of the content disappeared giving a pale orange color. This measures the free acidity. To the content titrated above, 2 drops of phenolphthalein indicator was added and titrated further against 0.01M NaOH till a faint pink color was obtained. The value obtained was the measure of the total acidity Kulkarni *et al.* (2011). This method was also used for fractions obtained from column chromatography.

Calculation: Free and total acidities of 1ml of gastric juice sample corresponds Xml of 0.01M NaOH. Free acidity of 10ml gastric juice sample will correspond to Xml of 0.1M NaOH i.e.,10X units of free or total acidity is the ml of 0.1N NaOH required for titration of 10ml of gastric juice sample and is equal to milliequivalent/litre.

3.3.2. **Estimation of gastric wall mucus**

Gastric wall mucus was determined according to the modified procedure of Corne *et al.* (1974). The glandular segments of the stomach was excised and weighed. Each segment was transferred immediately to 10ml of 0.1% w/v Alcian blue solution (in 0.1M sucrose solution buffered with 0.05 ml of sodium acetate at pH 5). Tissue was stained for 2 hr in Alcian blue and excess dye was removed by two successive rinses

with 10 ml of 0.25 M sucrose, first for 15 and then 45 minutes. Dye complexes with the gastric wall mucus were extracted with 10ml of 0.5 M $MgCl_2$ which was shaken intermittently for 1 min at 30 min intervals for 2 h. Four milliliter of blue extract was shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of aqueous layer was recorded at 580 nm. The same procedure was used to fractions obtained from column chromatography.

3.3.3 **Superoxide Dismutase (SOD) Determination**

Superoxide Dismutase was determined according to the method described by Fridovich (1989).

Principle: The ability of superoxide dismutase to inhibit auto oxidation of adrenaline at pH 10.2 forms the basis of this assay.

Procedure: Gastric tissue homogenate of 0.1ml was diluted in 0.9ml of distilled water to make 1:10 dilution of microsome. An aliquant mixture of 0.2ml of the diluted microsome was added to 2.5ml of 0.05M Carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contained 2.5ml of 0.05M Carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of distilled water. Absorbance was measured over 30s up to 150s at 480nm.

Calculations:

Increase absorbance per minute = $(A_5 - A_1)/2.5$

$$\% \text{ inhibition} = 100 - \frac{\text{Increase absorbance for substrate}}{\text{Increase in absorbance of blank}} \times 100$$

3.3.4 **Catalase Activity Determination**

The activity of catalase from the stomach tissue of the rats was determined according to the method of Aebi (1984).

Principle:

Protein- Fe^{3+} + H_2O_2 $\longrightarrow$ Protein-Fe^{2+}-OOH + H_2O

Protein-Fe^{3+}-OOH + H_2O $\longrightarrow$ Protein-Fe^{3+}-OH + H_2O + O_2

The overall reaction: $2H_2O_2$ $\longrightarrow$ $2H_2O + O_2$

Procedure: Tissue homogenate of about 10µl was added to 2.8ml of 50mM potassium phosphate buffer (pH 7.0) .The reaction was initiated by adding 0.1ml of fresh 30mM H_2O_2 and the decomposition rate measured at 240nm for 2 min on a spectrophotometer. A molar extinction coefficient of 0.041mm/cm was used to calculate catalase activity.

Catalase activity = Absorbance/E x Protein conc. (mg/ml).

3.3.5 **Assay of Glutathione Concentration**

Reduced glutathione concentration measurements was done according to Ellman (1959) as described by Rajagopalan *et al.* (2004).

Principle: The sulphydryl group of GSH reacts with DTNB and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulphide, GSTNB is reduced by GSH reductase to recycle more GSH.

Procedure:

To 150µL of gastric tissue homogenate (in phosphate-saline pH 7.4), 1.5ml of 10% TCA was added and centrifuge at 1500g for 5min. Then 1ml of the supernatant was treated with 0.5 ml of Ellman's reagent and 3ml of 0.2M phosphate buffer pH 8.0

added. The absorbance was read at 412nm. The quantity of GSH was obtained from the graph of the GSH standard curve.

3.4.0 Purification of aqueous fraction: Aqueous fraction of *Ziziphus abyssinica* roots was subjected to thin layer chromatography and column chromatography

3.4.1. **Thin Layer Chromatography (TLC).**

Preliminary TLC was carried out for selection of the best solvent system for column development. The method used was that of Abbot and Andrew (1970).

Principle: This involves separation of components based on relative solubility of compounds between the sorbent (stationary phase) and the solvent (mobile phase). Compounds that are more soluble in the mobile phase will migrate up the plate to a greater extent than components that are more soluble in the stationary phase.

Method: The content of each beaker was spotted on the TLC plate using capillary tube. The plate was then placed into a tank with sufficient suitable solvent to wet the lower edge of the plate before the pencil line (1cm from the bottom of the plate) and covered with glass lid. The solvent ascended to about ¾ of the length of the strip. Then the strip was removed and allowed to dry. The retention factor was calculated as:

$$R_F = \frac{\text{Compound distance from origin}}{\text{Solvent front distance from origin}}$$

3.4.2 **Column Chromatography:**

Principle: In column chromatography, separation is achieved based on relative migration rates of components effected by their individual affinities for the sorbent, and separation occurs when one compound is more strongly adsorbed by the sorbent than other components.

Method: In the setting up of column chromatography, the lower part of the column was stocked with glass wool with the aid of glass rod. Slurry was prepared by mixing sephadex and ethyl acetate which was used to pack the column to one-third its length. Powdered residue of aqueous fraction was mixed with activated sand and placed over the slurry in the column. Cotton wool was placed there after followed by solvents of increasing polarity.

3.5.0 **Antiulcerogenic Study of the Fractions From Column Chromatography: Grouping**

Twenty five rats were used for this experiment. The rats were grouped into five groups of five rats each as follows:

Group I: Rats were given food and water (not treated with either fraction).This group served as control group.

Group II: Rats were treated with (25mg/kg b.w.) of F_A and 1ml of 95% ethanol administered orally.

Group III: Rats were treated with (25mg/kg b.w.) of F_B and 1ml of 95% ethanol administered orally.

Group IV: Rats were treated with (25mg/kg b.w.) of F_C and 1ml of 95% ethanol administered orally.

Group V: Rats were treated with (25mg/kg b.w.) of F_D and 1ml of 95% ethanol administered orally.

3.6 **Histopathological Studies:**

Gastric tissue samples from each group were fixed in 10 per cent formalin. The formalin fixed specimens were embedded in paraffin, sectioned and stained with haematoxylin and eosin. The histological sections were examined under microscope and then photographed at magnification 400.

3.7 **Statistical Analysis**:

Data obtained was expressed as mean ± standard deviation and analyzed using SPSS 17. The significance among groups was determined by ANOVA and LSD post hoc test for multiple comparisons**.** Values of $p<0.05$ were regarded as statistically significant.

CHAPTER FOUR

4.0 RESULT

4.1 Effect of Ziziphus abyssinica root extracts on lesion index

Rats that were given ethanol showed extensive lesions in the gastric portion of the stomach. In contrast, oral administration of the extracts at 300mg/kg and 600mg/kg body weight for fourteen days before ulcer induction lowered the lesion index values significantly ($P<0.05$) and aqueous extract had the highest protective effect as shown in Table 4.1. There were no gastric lesion in rats that were pretreated with either the standard (cimetidine) drug or the aqueous extract pretreated rats, but there were hemorrhages in some rats.

The effect of *Ziziphus abyssinica* root extracts on lesion index of animals administered with 1ml of 95% ethanol is shown in Table 4.1 while Fig. 4.2 shows their respective percentages of protection. From the figure, rats administered with standard drug and aqueous extract group (300mg/kg) showed highest percentage protection (95%) followed by aqueous extract of 600mg/kg with percentage protection of 93% but the difference between the two is not statistically significant. This is supported by the histopathological observations in plate 4.1. From the plate (histological picture), all extracts pretreated groups showed changes in the cytoarchitecture of gastric mucosa after ethanol administration, with aqueous extract having the highest protective effect.

4.2 ***Determination of LD_{50} of Aqueous extract of Z. abyssinica roots***: The LD_{50} of the aqueous root extract of *Ziziphus abyssinica* was found to be greater than 5000mg/kg

body weight. However a reduced reaction to noise, slow movement and resting at the corner of cages was observed after administration of the extract with no any sign of toxicity such as increased breath rate, convulsion, shivering and feeling of cold, loss of consciousness, stomach pain, heart beat rate etcetera.

Table 4.1: Effect of *Z. abyssinica* root extracts on lesion index

Treatment	Dose (mg/kg)			
	0	100	300	600
Control	□	□	□	□
Ulcer Control	4.2±0.68[a]	□	□	□
Hexane	□	□	3.2±1.16[b]	3.0±0.89[b]
Ethyl acetate	□	□	2.1±0.58[c]	2.3±0.68[c]
Methanol	□	□	1.4±0.78[d]	1.6±0.48[d]
Aqueous	□	□	0.3±0.24[e]	0.2±0.24[e]
Cimetidine	□	0.2±0.24	□	□

Values are mean ± Standard Deviation for 5 animals in each group. Different superscript in the same column are Significantly at (P<0.05) by using analysis of variance.

a = Not significantly different from ulcer control at p<0.05

b = Significantly different from ulcer control group but the same with hexane pretreated group at p<0.05

c = Significantly different from hexane pretreated group at p<0.05, the same with ethyl acetate pretreated group

d = Significantly different from ethyl acetate pretreated group at p<0.05, the same with methanol pretreated group

e = Significantly different from methanol pretreated group, the same with standard control group at p<0.05

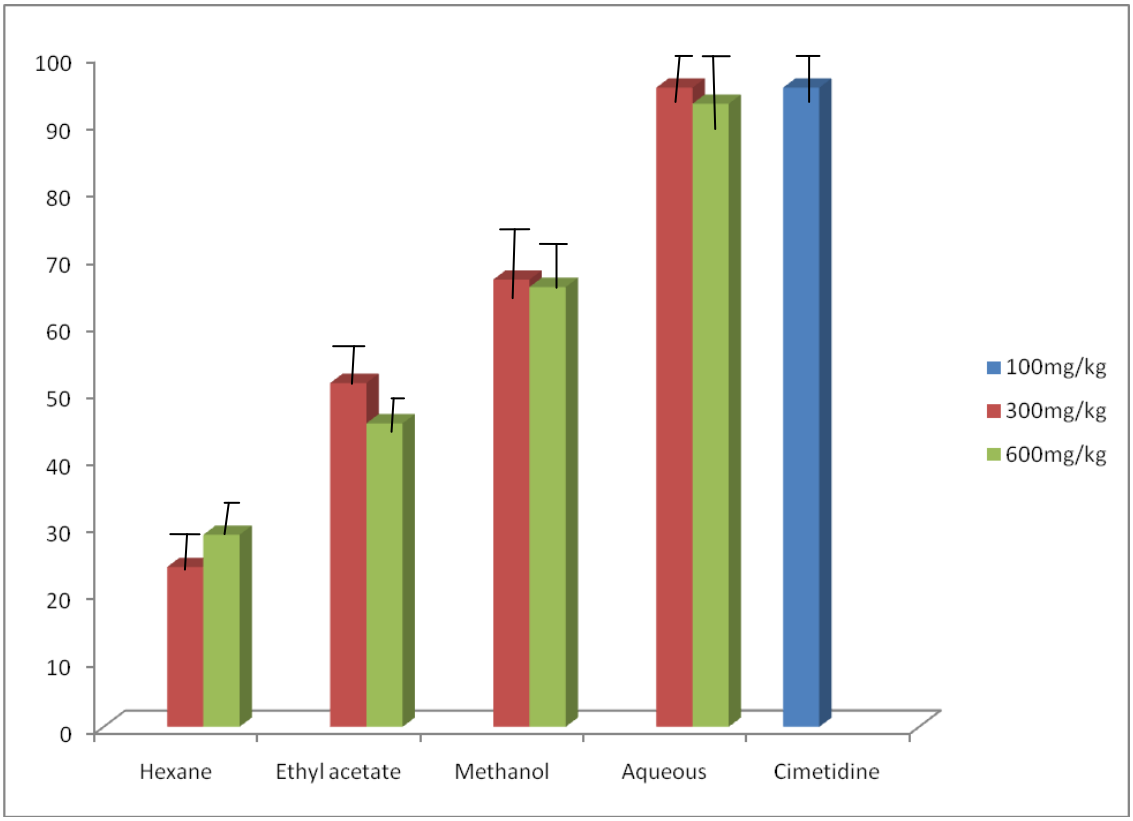


Figure 4.1: Percentage protection of *Z. abyssinica* root extracts in comparison with control in ethanol induced ulceric rats.

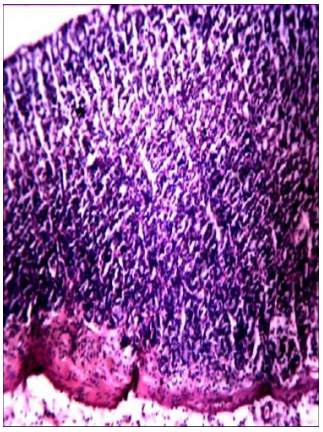


Plate4.1a: normal features of mucosa

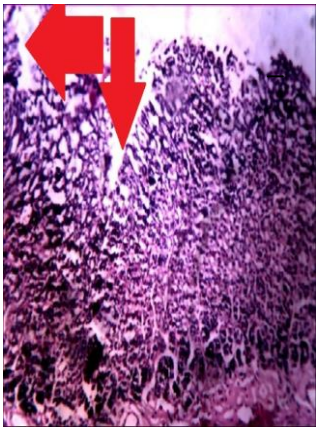


Plate4.1b: slight mucosa epithelial changes

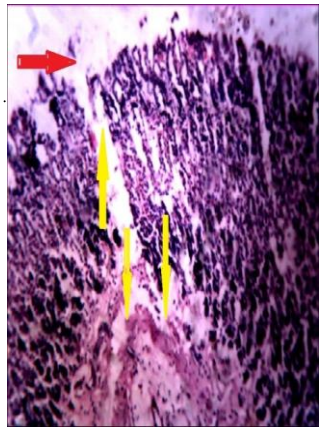


Plate 4.1c: ulceration of mucosa lining

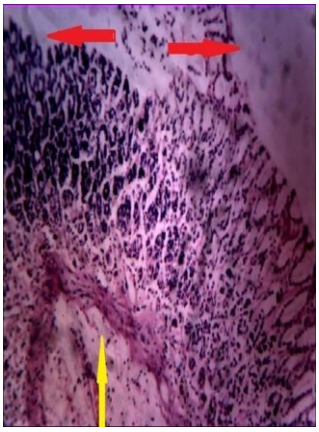


Plate4.1d: Intense necrotic changes to mucosa epith.

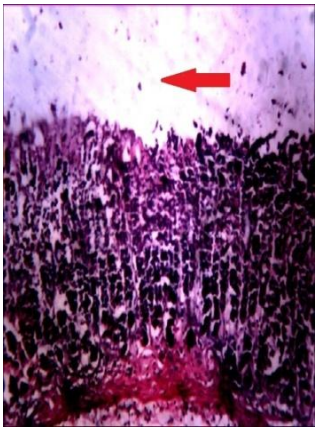


Plate 4.1e: Necrotic changes to apical epithelium

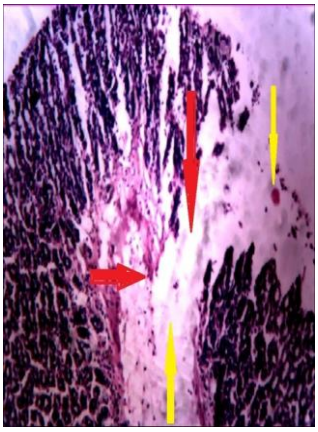


Plate 4.1f: Ulceration & necrosis of mucosa

Plate 4.1: Histological examination of control and experimental animal pretreated with *Ziziphus abyssinica* root extracts 300mg/kg body weight. Standard control group (*4.1a*); aqueous extract pretreated group (*4.1b)*; methanol extract pretreated group (*4.1c*); ethyl acetate extract pretreated group (*4.1d*); hexane extract pretreated group (*4.1e*); ulcer control group (*4.1f*). The red arrow in the figure shows necrosis; the yellow arrow shows ulceration of gastric tissue. Magnification x 400.

4.3 Antiulcer activity of aqueous and butanol fractions:

Table 4.2 shows the effect of butanol and aqueous fractions of *Z. abyssinica* roots on gastric secretion in ethanol induced ulceric rats. From the Table, aqueous fraction showed significant antiulcer activity as it significantly ($P<0.05$) reduced the volume of the juice secreted; increased pH, and decreased acidity levels. Also, mucus content has significantly increased ($P<0.05$) (Fig. 4.2) with significant decrease in lesion index value (Table 4.2).This was supported by results from histological examination of of gastric mucosal tissue sections of control and experimental rats pretreated with 100mg/kg and 200mg/kg aqueous and butanol fractions (*Plates 4.2a-f*).

From the plates, control rats showed normal appearance of gastric mucosa with changes in the cytoarchitecture of the mucosa in the rat pretreated with fractions. There was slight change in the mucosal epithelium of the rats that were pretreated with 100mg/kg aqueous fraction; but there was necrosis and ulceration of mucosal epithelium in rats that were pretreated with 200mg/kg aqueous fraction. In animals pretreated with butanol fractions, there was intense necrosis and ulceration of mucosal epithelium in rats given 100mg/kg and ulceration of mucosa in rats given 200mg/kg body weight of butanol fraction. The quantities of the fractions after solvent partitioning were 0g for hexane; 0.76g for ethyl acetate; 21.3g for butanol and 37.4g for the remaining aqueous fraction.

The effect of *Ziziphus abyssinica* root aqueous extract fractions on catalase, superoxide dismutase enzymes and glutathione concentration are shown in Fig. 4.3. From the Figure, aqueous fraction (100mg/kg b.w.) showed highest activities of CAT, followed by 200mg/kg aqueous fraction but the difference between the two is not significant when compared with ulcer control group. There was reduced SOD activities in all the

rats pretreated fractions compared to ulcer control group and an increase glutathione concentration in aqueous fraction pretreated rats as compared to ulcer control group.

Table 4.2: Effect of Aqueous and Butanol fractions on Gastric secretion Parameters

Treatment	Dose (mg/kg)	gastric juice (mL)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)	Ulcer Index Protection (mm)	(%)
Control	□	1.54±0.22[a]	3.52±0.14[a]	28.0±3.80[a]	65.2±1.92[a]	_	_
Ulcer control	□	2.24±0.56[b]	2.53±0.38[b]	82.0±21.4[c]	174.0±2.21[d]	4.2±0.68[c]	□
Cimetidine	100	1.02±0.40[c]	4.28±0.32[c]	28.0±7.48[a]	72.2±2.40[a]	0.2±0.09[a]	88.1
Aqueous	100	1.82±0.26[a]	4.08±0.24[c]	30.0±4.00[a]	70.4±1.46[a]	1.8±0.26[b]	52.6
	200	2.10±0.24	4.64±0.12[c]	46.0±15.50[b]	63.6±1.28[a]	2.1±0.24[b]	57.1
Butanol	100	2.26±0.26	3.62±0.48[a]	54.0±10.20[b]	135.2±9.30[b]	2.26±0.26[b]	28.6
	200	2.28±0.35	3.68±0.52[a]	44.0±5.22[b]	154.6±2.14[c]	2.28±0.35[b]	18.7

Values are mean± Standard Deviation. Different superscript in the same column are statistically significant at (P<0.05).

a = No statistical significance with normal control at $p<0.05$

b = No statistical significance with ulcer control group but significantly different from normal control at $p<0.05$

c = No statistical significance with standard control group but significantly different from normal control group at $p<0.05$

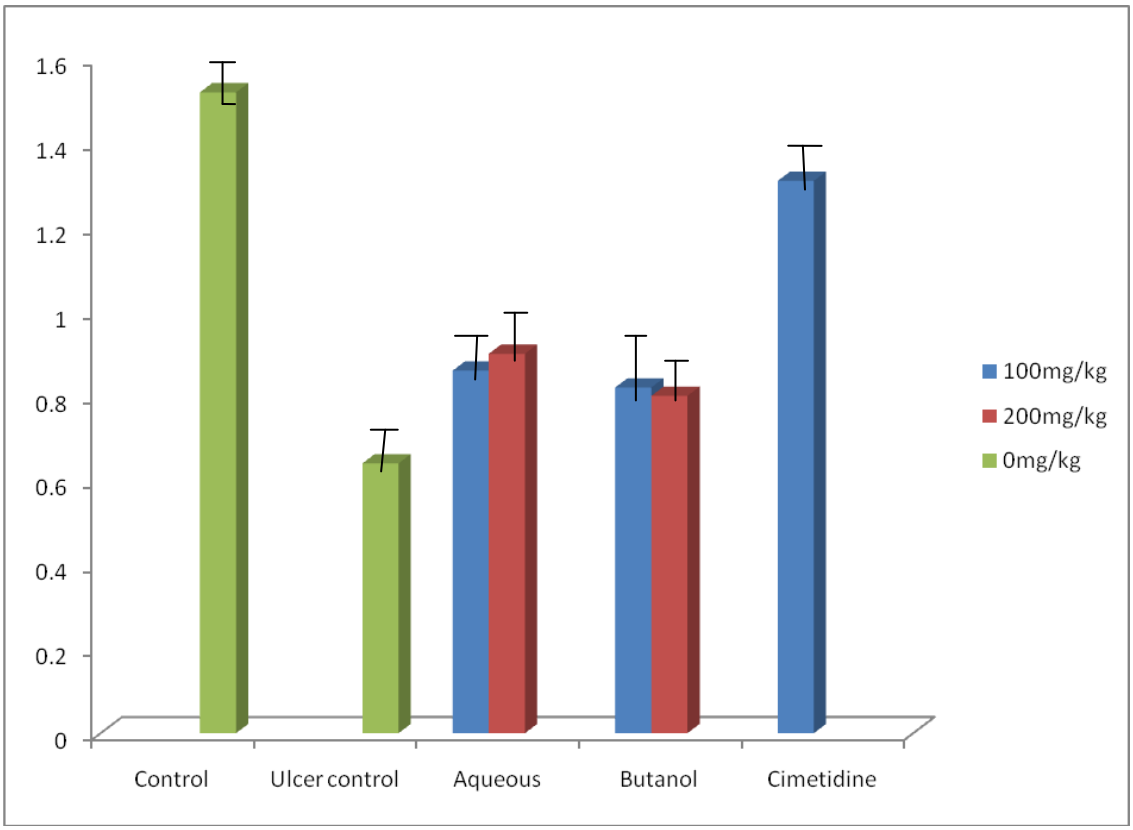


Figure 4.2: Effect of *Z. abyssinica* aqueous extract fractions on adherent mucus content of control and experimental animals

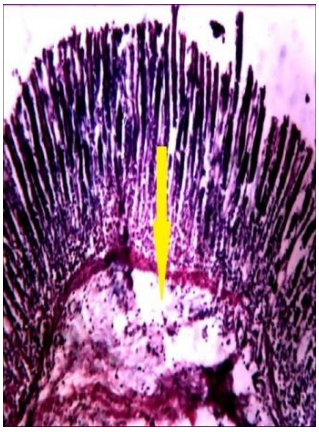


Plate4.2a: Slight mucosa epithelial change

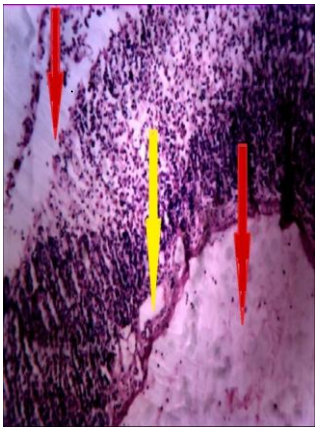


Plate 4.2b: mucosa necrosis with ulceration

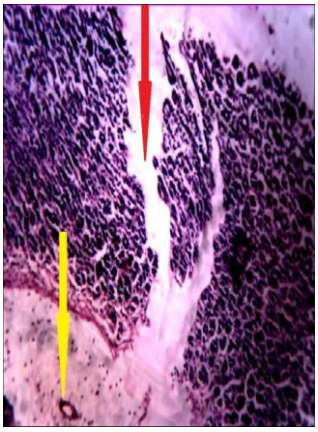


Plate4.2c: Intense necrosis & ulceration

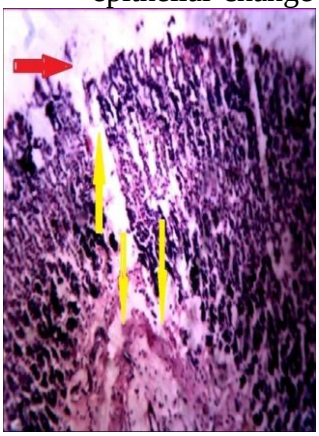


Plate 4.2d: ulceration of mucosa epithelium.

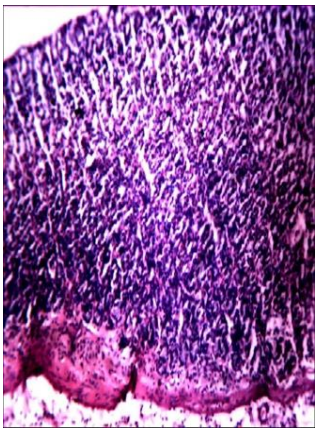


Plate 4.2e: Normal features of mucosa

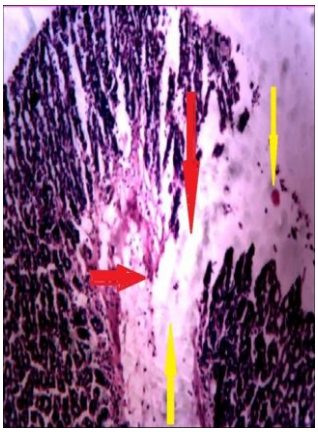


Plate 4.2f: Intense ulceration & necrosis of mucosa.

Plate 4.2: Histological examinations of gastric mucosal tissue sections of control and experimental animals pretreated with 100mg/kg and 200mg/kg of butanol and aqueous fractions for ten days. Aqueous fraction pretreated group 100mg/kg (4.2a); aqueous fraction pretreated group 200mg/kg (4.2b) butanol fraction pretreated group 100 mg/kg (4.2c); butanol fraction pretreated group 200mg/kg (4.2d); standard control group (4.2e); ulcer control group (4.2f)The red arrow in the figure shows necrosis of the mucosa; the yellow arrow shows ulceration of gastric tissue. Magnification X 400.

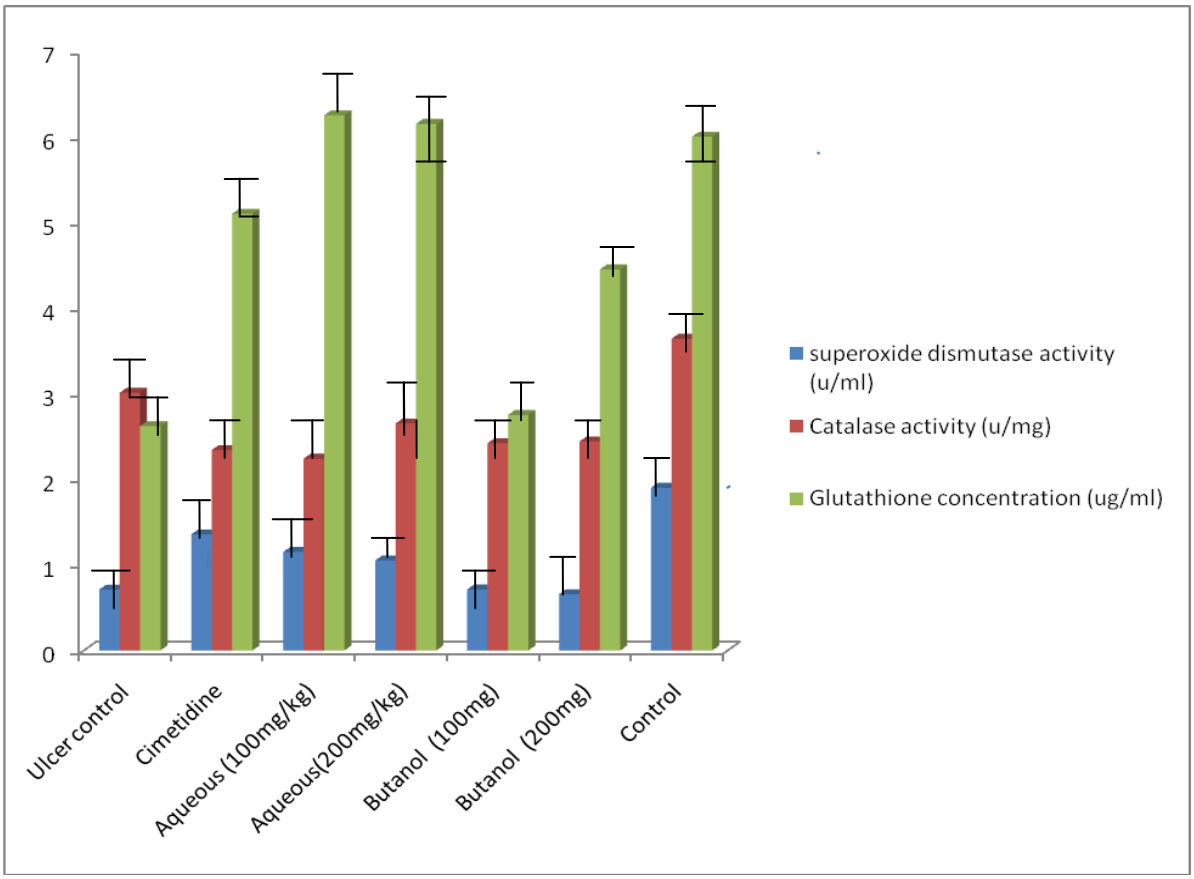


Figure 4.3: Effect of *Ziziphus abyssinica* root fractions on catalase, superoxide dismutase enzymes and glutathione concentration.

4.4 **Column chromatography**: Column chromatography of the aqueous fraction of *Ziziphus abyssinica* roots was carried out. Fourteen fractions were collected at the rate of 40ml/20minutes. Out of the fourteen fractions, six beakers were found to contain some isolates and coded as F_3, F_4, F_5, F_6, F_7 & F_8, while the remaining beakers; F_1, F_2, F_9, F_{10}, F_{11}, F_{12}, F_{13} and F_{14} were empty. These fractions were subjected to TLC using ethyl acetate-methanol in the ratio of 1:1, fractions with the same R_f value were pooled to make four (4) fractions. $F_3 + F_4 = F_A$; $F_5 = F_B$; $F_6 + F_7 = F_C$ and $F_8 = F_D$.

Table 4.3*: TLC properties of colum*n *fractions from the Aqueous extract of Ziziphus abyssinica roots*

Fractions	R_f	TLC coloration
F_A	0.766	Dark brown
F_B	0.215	Brown
F_C	0.771	Brown
F_D	0.613	Reddish brown

Eluent: Ethyl acetate and methanol (1:1)



Figure 4.4: TLC profile of aqueous column fraction. Solvents used: *Ethyl acetate-Methanol* (1:1).

4.5 **Effect of *Z. abyssinica* root Column fractions on gastric secretion parameters**: The effects of Column fractions from *Ziziphus abyssinica* root aqueous extract using ethanol induced gastric ulcer in rats are shown in Table 4.5. From the Table, each fraction has inhibited ulcer formations via reduced gastric juice volume, acidity levels with increase in pH. Consequently, from the histological examination of gastric mucosal tissue sections, control group (*Plate4.3a*) showed normal appearance of the mucosa; F_A treated group showed slight mucosa epithelium change (*Plate 4.3b*); F_B treated rats showed necrosis of mucosa epithelium (*Plate 4.3c*); F_C treated rats (*Plate 4.3d*) showed minimal lesions with slight mucosa necrosis while F_D (*Plate 4.3e*) showed intense ulceration and necrosis of the mucosa.

Table 4.5: Effect of *Ziziphus abyssinica* root column fractions in gastric secretion and ulcer index.

Treatment	Dose (mg/kg)	Gastric Juice (mL)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)	Mucus (mg/g)	Ulcer Index (mm)	Protection (%)
Control	-	2.1±0.27[a]	3.24±0.16	48.3±3.42[a]	84.6±4.22[a]	1.02±0.04[a]	-	-
F_A	25	1.5±0.53[c]	3.31±0.42	60.2±2.42[b]	104.2±6.34[a]	0.96±0.16[a]	1.5±0.66	64.3
F_B	25	1.8±0.14[ab]	3.10±0.24	64.8±4.60[b]	141.2±13.43[b]	0.72±0.20[b]	2.3±0.46	45.2
F_C	25	1.4±0.34[c]	3.35±0.33	42.6±1.66[a]	76.2±2.43[a]	1.22±0.14[a]	1.2±0.26	71.4
F_D	25	1.9±0.28[a]	3.06±0.01	73.8±5.60[c]	152.0±9.84[c]	0.64±0.12[c]	1.9±0.38	54.7

Values are mean ± SD for 5 animals in each group. Different superscript in the same column are significantly different at ($p<0.05$).
a = No statistical significance with normal control at $p<0.05$
b = Statistically significant with normal control at $p<0.05$
c = Significantly different from control group and other groups at $p<0.05$



Plate4.3a: Normal features of mucosa epithelium

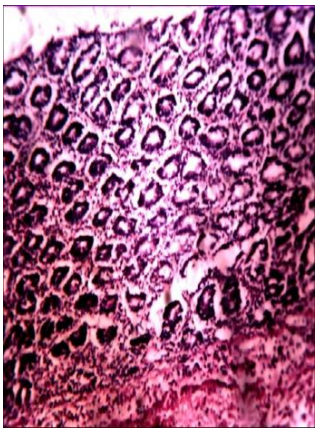


Plate4.3b:Slight mucosa epithelial changes

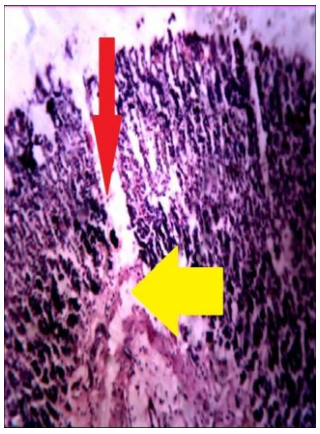


Plate4.3c: Necrosis of epithelium

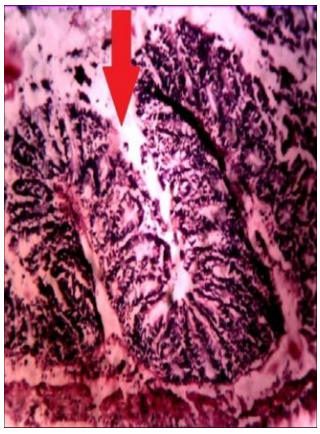


Plate 4.3d: Slight necrosis of mucosa ulceration

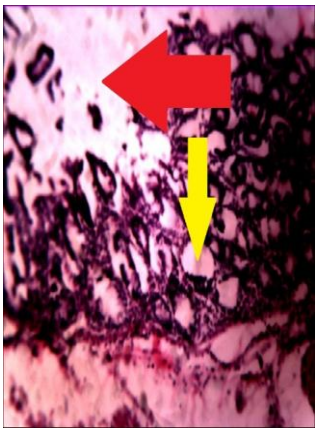


Plate 4.3e: Intense necrosis & deep ulceration

Plate 4.3: Histological examination of control and experimental rats treated with 25mg/kg body weight of fractions (F_A – F_D) from aqueous extract after column chromatography.

Control group (Plate 4a); F_A treated group (Plate 4b); F_B treated group (Plate 4c); F_C treated group (Plate 4d); F_D treated group (Plate 4e). The red arrow in the figure shows necrosis; the yellow arrow shows ulceration of the gastric mucosa. Magnification X 400.

CHAPTER FIVE

5.1 DISCUSSION:

Ethanol is considered one of the agents that induce intense gastric ulcers as it promotes serious disturbances in gastric mucosal blood flow and mucus production in the gastric lumen (Moratto *et al.*, 1999). In this study, all extracts have significantly ($P<0.05$) reduced the development of ulcers, with aqueous extract having the highest protective effect (95%) as it showed reduced lesions formation in the gastric tissue of the rats pretreated this extract.

Considering the data obtained from ethanol induced model, the observation that the response of the extracts decreased with increased dose is consistent with findings of studies on some other plants as reported by some authors (Pandit *et al.,* 2000). Such a less response at higher dose is common with indigenous plants and this observation could be due to therapeutic windows effect as suggested by Zakaria *et al*. (2011) .It could also be that the effect of the extract has reached its maximum level at a dose of 300mg/kg, the lowest dose used in this study for the extracts.

In histological study, pretreatment with aqueous extract was found to preserve the functional cytoarchitecture of the entire mucosa with some pathological changes. The extract therefore showed maintenance of mucosa. These findings confirm cytoprotective nature of the extract (Jainu and Devis, 2006). Hence this extract was partitioned with hexane, ethyl acetate and butanol. Because the yield of hexane and ethyl acetate was very small and will not be enough for *in vivo* studies, we used only the butanol and the remaining aqueous fraction.

Inhibition of gastric acidity is one of the important protective factors against gastric ulcer. In this study, aqueous fraction (100mg/kg) exerted a significant ($p<0.05$) protective effect as it significantly reduced gastric juice volume, increased pH, reduced acidity levels when compared with ulcer control group. However, non significant increase in juice volume was observed in rats pretreated 200mg/kg b.w. aqueous fraction but a significant increase in pH and decreased acidity levels. The result of this study suggests that the aqueous fraction has the highest antisecretory effect (Jainu *et al*., 2006). Gastric acid secretion is stimulated by the release of histamine from enterochromaffin-like cells in the oxyntic glands. The reduction of gastric acidity and gastric juice volume could be attributed to antihistamine effect of the fraction thereby reducing the acidity of the juice.

Mucus secretion is a crucial factor in the protection of gastric mucosa from the gastric lesions and has been regarded as an important defensive factor in the gastric mucosa barrier. The results of this study showed increased levels of adherent mucus content of gastric tissue in animals pretreated with aqueous fraction indicating its cytoprotective action on experimentally induced ulcer.

Free radicals contribute to the pathogenesis of ulcer. Plant based antioxidants have been shown to delay or inhibit the onset of gastric ulcers and carcinoma. In the present study, ethanol induced ulcer is shown to deplete the enzymatic antioxidants in gastric tissues. The antioxidants levels are significantly maintained in test drug treated rats. Experimental alcoholism has been reported to cause both sympathetic and parasympathetic stimulation of stomach leading to hypoxia and ischemia like conditions to increase the level of free radicals which oxidize cellular components (Chatuverdi *et al*., 2007).

Superoxide dismutase (SOD) plays an important role in providing gastroprotection partly by preventing oxidative damage. SOD destroys the highly reactive radical superoxide ($O_2^{\bullet -}$) by converting it into less reactive peroxide (H_2O_2) which can be destroyed by catalase (CAT) reaction. CAT is a highly reactive enzyme that reacts with H_2O_2 to form water and molecular oxygen. GSH and related enzymes in tissues have been proposed as potential chemopreventive agents due to their antioxidant and detoxification properties (Pohle *et al*., 2001). In the present study, rats pretreated with *Z. abyssinica* aqueous fractions showed a significant increase in CAT and SOD activities and an increase in glutathione concentration which might be attributable to its cytoprotection and antioxidant activities. The decreased CAT activities observed in butanol fractions could be due to inhibitory effect of the fractions on synthesis of the enzyme (CAT) leading to increased production of hydrogen peroxide which is known to cause oxidative damage to membrane lipids, nucleic acids and proteins leading to mucosa damage. The results of this study showed that *Ziziphus abyssinica* aqueous root fractions restored the activities of antioxidant enzyme of CAT, SOD and GSH concentration compared to ulcer control group. This agrees with the findings of Saranya and Geetha (2010).

The histological examination of rats pretreated both butanol and aqueous fractions showed changes in the appearance of the mucosa with distorted mucosal epithelium. Protection against these histopathological changes was observed by maintenance of mucularis mucosa and apparent epithelialization only in standard control group (cimetidine).

The antiulcer activity of roots of *Ziziphus abyssinica* extracts might be attributable to the presence of biological compounds such as flavonoids, alkaloids, tannins and other

polyphenols present in the extracts. Alkaloids have been shown to have gastroprotective and ulcer healing activity by inhibition of acid secretion and gastric motility, stimulation of alkali/mucus secretions, increase mucosal blood flow (Pandit *et al.*, 2000). Tannins precipitate microproteins at the site of injury forming protective pellicle that prevents absorption of toxic substances and promote resistance to the action of proteolytic enzymes; and therefore promote tissue repair, acid secretion inhibition, antioxidant and *anti Helicobacter pylori* activity (Vasconcelos *et al.*, 2008). Some flavonoids have been shown to increase the mucosal content of prostaglandins and mucus in gastric mucosa showing cytoprotective effect against necrotic agents (Alanko *et al*., 1999). These plant constituents present in *Ziziphus abyssinca* extracts might have the ability to protect against ulceration induced by ethanol.

In column chromatography, fraction c became the most bioactive components of the extract when it protected the stomach to some extent from experimentally induced ulcerative lesions. Besides, the volume of the gastric juice has significantly decreased compared to control group; free and total acidities have also decreased significantly, with statistically insignificant increase in pH. These effects show the cytoprotective nature of F_C which had increased gastro-protective effects against toxic injury caused by ethanol in the experimental rats. The result from this study suggests that the antiulcer effects of the extract could therefore be synergistic. From histology of the fractions, there were changes in the structure of the stomachs of the rats treated fractions, with F_C showing the highest protection against lesions induced by ethanol with slight structural changes of mucosa epithelium. Minimal ulcer lesions were produced in the stomach of rats that were treated with F_C as compared with other fractions. This showed that F_C is the most potent bioactive fraction of the extract.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 **Summary**

- Pretreatment with the extracts of roots of *Ziziphus abyssinica* has significantly ($P<0.05$) lowered the lesion index (not dose dependent) with aqueous extract having the highest effect. The lesion index being 3.2±1.16 and 3.0±0.89 for hexane extract; 2.1±0.58 and 2.3±0.68 for ethyl acetate extract; 1.4±0.78 and 1.6±0.48 for methanol; 0.2±0.24 and 0.3±0.24 for aqueous extract; all at the dose of 300mg/kg and 600mg/kg body weight respectively.

- The LD_{50} of the aqueous extract of roots of *Ziziphus abyssinica* was found to be greater than 5000mg/kg body weight with no any toxicity sign.

- Aqueous fraction derived from aqueous extract showed the highest antiulcer effects as it significantly lowered the lesion index, reduced the volume of gastric juice, total acidity and increase the pH and mucus content of the gastric tissue.

- The lesion index values of pretreatment with aqueous and butanol fractions are 1.8±0.26 and 2.1±0.24 for 100 mg/kg b.w and 200mg/kg b.w. aqueous fraction; 2.26±0.26 and 2.28±0.35 for 100 mg/kg and 200 mg/kg butanol fractions respectively.

- The pH and juice volume were 3.52±0.14 and 1.54±0.22 respectively for control group; 2.53±0.38 and 2.24±0.56 for ulcer control group; 1.02±0.40 juice volume and 4.28±0.32 pH for standard control group.

- In aqueous fractions pretreated groups, the juice volume was 1.82±0.26 and 2.10±0.24 for 100mg/kg and 200mg/kg b.w. aqueous fraction; 2.26±0.26 and 2.28±0.35 for butanol fractions. The pH was 3.62±0.48 and 3.68±0.52 for 100mg/kg and 200mg/kg butanol fractions respectively; 4.08±0.24 and 4.64±0.12 aqueous fraction respectively.

- Of the four fractions obtained, F_c showed the highest antiulcer effect and therefore considered the most bioactive fraction of *Z. abyssinica* roots.

6.2 **Conclusion**

Ziziphus abyssinica root extracts showed significant antiulcer activity in experimentally induced ulcer rat model with aqueous extract and aqueous fraction having the highest protective effect. The activity was not dose dependent.

The present finding suggests that *Z. abyssinica* roots promotes ulcer protection by the decrease in ulcer index, gastric secretions, increased mucus, catalase and superoxide dismutase activities and an increase in glutathione concentration.

In column chromatography, F_c showed the highest protective effect and therefore considered the most bioactive fraction. The result shows that the gastroprotective effect of *Z. abyssinica* roots was due to its antioxidant, antisecretory and cytoprotective effects.

6.3 **Recommendations**

I. Since *Z. abyssinica* possesses anti-bacterial activities, it may be necessary to study its effect on *Helicobacter pylori* activities (*in vitro*).

II. GCMS and NMR of the active fractions should be carried out to elucidate the structure of the active compound present in *Z. abyssinica* as well as its characterization.

REFERENCES

Abbot D. and Andrew R.S. (1970). An introduction to chromatography, 2nd ed. Longman press, London, Pp. 77-78.

Aebi, H.E. (1984). Catalase In: Methods of enzymatic analysis, Bergmer H.U (Ed.) vol.3 Weinheim, Germany, Verlag Chemie pp. 273-286.

Alanko J, Riutta A, Holm P, Mucha I, Vapatalo H and Metsa-Ketela T. (1999). Modulation of arachidonic acid metabolism by phenols: relation to their structure and antioxidant/proxidant properties. *Free Radical Biology and Medicine*, 26(2), 193-201.

Albuquerque, U.P, Monteiro, J. M,and Aroujo, E. L.(2005). Uma abordagem da quimica a Ecologia. *Quim. Nova*, 28, 892-896.

Alvarez, E.O. (2007). "The role of histamine on cognition." *Behavioural Brain Research*, 199(2), 183-189.

Aniagu S.O., Binda L.G., Nwinyi F.C., Orisadipe A., Amos S., Wambebe C., and Gamaniel K. (2005). Anti-diarrheal and ulcer-protective effects of the aqueous root extract of *Guiera senegalensis* in rodents. *Journal of Ethnopharmacology*, 97, 51-56.

Azam-Ali S., Bonkoungou E., Bowe C., deKock C., Godara A., and Williams J.T.(2012). Berry and other jujubes of *Ziziphus* species. *International Journal of Plant Science Research*, 3(3), 818-821.

Babincova M., Schronerova K. and Sourivong P. (2008). Antiulcer activity of water extract of *Scoparia dulcis. Fitoterapia*, 75, 587-588.

Bai, Y. F, and Xu, H.(2000). Protective action of piperine against experimental gastric ulcer. *Acta pharmacology*, *Sin* 21, 357-359.

Bandyopadhyay, D, Biswas, K., Bhattacharyya, M., Reiter, and R.J, Banerjee, R.K. (2001). Gastric toxicity and mucosal secretion induced by oxygen-derived reactive species: Protection by melatonin. *Current Molecular Medicine*, 1, 501-513.

Bansal V.K, and Goel R.K.(2012). Gastroprotective effect of *Acacia nilotica* young seedless pod extract: role of polyphenolic constituents. *Asian Pacific Tropical Medicine*, 5, 523-528.

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

Brand M.D, Affourtit C, and Esteves T. C. (2004). Mitochondria superoxide: Production, Biological effect and activation of uncoupling proteins. *Free Radical Biology and Medicine*, 37, 755-767.

Cartea, M. A., Francisco, M., Soengas, P., and Velasco, P. (2011). Phenolic compounds in *Brassica vegetables*. *Molecules,* 16, 251-280.

Chattophadhyay, I., Bandyopadhyay, U., Biswas, K., Maity, P., and Banerjee, R.K.(2006).Indomethacin inactivates gastric peroxidase to induced reactive oxygen Mediated Gastric mucosal injury and curcumin protects it by preventing peroxidase inactivation and scavenging reactive oxygen. *Free Radical Biology* and *Medicine,* 40, 1397-1408.

Chaturvedi A., Kumar M.M., Bhawani G., Chaturvedi H., Kumar M., and Goel R.K. (2007). Effect of ethanolic extract of *Eugenia jambolana* seeds on gastric ulceration and secretion in rats. *Indian Journal of Physiology and Pharmacology,* 15, 131-42.

Corne S.J., Morriessey S.M., and Woods R.J. (1974). Proceedings: A method for the quantitative estimation of gastric mucus. *Journal of Physiology,* 242, 116-117.

Cragg, G. M., Newmann, D.J. and Snader, K.M. (1997). Natural products in drug discovery and development. *Journal of Natural Product*, 60, 52-60.

Cryer B., and Spechler S.J. (2006). Peptic Ulcer Disease. In: Fried L.S. Brandt L.J, Sleisengerand *Fordtran's Gastrointestinal and Liver Disease 8th edition,* Philadelphia, Pp. 1089-1110.

Chikamai B., Lyog-Matig O. and Mbogga M. (2004). [Review and Appraisal on the status of Indigenous fruits in Eastern Africa. A Report prepared *for IPGRI-SAFORGEN* in *The frame work of AFREA/FORNESSA*] retrieved from *http:www.bioversityinternational.org/../*Review and synthesis of Indigenous fruits in East Africa.pdf.

David L.N. and Michael M. (2008). *Lehninger's Principles of Biochemistry. Fifth Edition*. W.H. Freeman and Co., pp.359.

Devi, K.R., Atluri J.B., and Reddi C.S. (1989). Pollination ecology of *Ziziphus mauritiana* (*Rhamnaceae*). Proceedings of the Indian Academy of Sciences. *Plant Sciences*, 99(3), 223-239.

Ellman G.D.(1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics,* 82, 70-77.

Ezike A.C., Akah P.A., Okoli C.O., Ezeuchenne N.A., and Ezeugwu S. (2009). *Carica Papaya* (paw-paw) Unripe Fruit May Be Beneficial in Ulcer. *Journal of Medicinal Food,* 12, 1268-1273.

Fridovich I. (1989). Method for the Determination of Superoxide Dismutase Activity in Tissues. *Journal of Biological chemistry,* 32, 102-106.

Gupta P., Nain P. and Sidana J. (2012). Oxidative stress induced ulcer protected by natural antioxidants. *International Research Journal of Pharmacy*, 3(5), 76-81.

Halliwell B. (1991). Reactive oxygen species in living systems: source, biochemistry and role in human diseases. *American Journal of Medicine,* 91, 14S-22S.

Hano J., Bugajsky J., Danek L. & Wantueh C. (1976). Nifedine versus cimetidine in prevention of stress induced gastric ulcer rats. *European Journal of Pharmacology,* 33, 277.

Haraguchi H., Yoshida N., Ishikawa H., Tamura Y., Mizutani K. & Kinoshita T. (2000). Protection of mitochondrial functions against oxidative stresses by isoflavans from *Glycyrrhiza glabra. Journal of Ethnopharmacology,* 52(2), 219-223.

Henriques, A.T., Limberger, R.P., Kerber, V.A., Moreno, P.R.H. (2004). In: *Farmacognosia*:*da planta ao medicamento*, 5ed., Simoes, C.M.O. Eds. Editoras of the Universidades Federais de Santa Catarina and Rio Grande do Sul: porto Alegre/ Florianopolis, Brazil, chapter 29, pp. 765-792.

Hirano T., nagashima R. and Hinohara Y. (2001). Selective binding of sucralfate to ulcer lesion in rats. *Gastroenterology*, 82(3):401-405.

Hiruma-Lima, C. A., Santos, L.C., Pellizon, C.H., Silveira, G.G., Vasconcelos, P.C.P., Vilegas, W., Souza Brito, A.R.M. (2006). *Qualagrandiflora*, a Brasilian cerrado Medicinal plant presents an important antiulcer activity. *Journal of Ethnopharmacology*, 104, 207-214.

Howden, C.W. and Hunt, R.H. (1994). Peptic Ulcer Disease. In: Lewis, J.H. (Ed.), A *Pharmacologic Approach to Gastrointestinal Disorders.* Williams and Wilkins, Maryland, USA, pp. 1-21.

Hunt, R.H. (2003). The gastrointestinal safety of the COX-2 selective inhibitor etoricoxib assessed by both endoscopy and analysis of upper gastrointestinal events. *American Journal of Gastroenterology*, 98, 1725-1733/ Article pubmed/ISI/Chempart.

Ishige K., Schubert D. and Sagara Y. (2001). Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. *Free Radical Biology and Medicine,* 30(4), 433- 466.

Jain, K.S., Shab, A.K., Bariwal, J., Shelke, S.M., Kale, A.P., and Jagtap, J.R. (2007). Recent advances in proton pump inhibitors and management of acid-peptic disorders. *Bioorganic Medical Chemistry*, 15, 1181-1205.

Jainu M., and Devi CSS. (2006). Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer model: Possible mechanism for the inhibition of acid formation. *Journal of Ethnopharmacology*. 104, 156-163.

Jainu M., Vijal Mohan K. and Devi CSS. (2006). Gastroprotective effect of *Cissus quadrangularis* extract in rats with experimentally induced ulcer. *Indian Journal of Medicinal Research,* 123, 799-806.

John D.V. (2005). Peptic ulcer disease and related disorders.In: *Harrison's Principles of Internal Medicine*, sixteenth ed. McGraw Hill, USA, pp. 1746-1762.

Kakei, N., Ichinose, M. and Tsukada, S. (1993). Omeprazole, a proton pump inhibitor, reduces the secretion, synthesis and gene expression of pepsinogen in the rat stomach. *Biochemistry, Biophysics Research*, 195, 997-1004.

Kanitkar U.K. (1969). A review on some Indian medicinal plants. *Journal of Medicinal Research India,* 3, 42-43.

Kent Lloyd K.C. and Debas H.T. (1994). Peripheral *regulation of gastric acid secretion*, In: Johnson L.R.(Ed.), *Physiology of the Gastrointestinal tract*. Raven Press, New York, pp. 1126-1185.

Khazaei M. and Salehi H.(2006). Protective effect of *Falcaria vulgaris* extract on ethanol induced gastric ulcer in rat. *Iranian Journal of Pharmacology and Therapeutics*, 5, 143-46.

Kikuko, A., Kyako I., and Susumi O. (1999). Antiulcer and Healing Effects of S-0509, a Novel CCK-B/ Gastrin Receptor Antagonist in Rats. *Digestive Diseases and Sciences,* 44(5), 879-888.

Kobata A., Kotani T., and Komatsu Y. (2007). Dual action of nitric oxide in the pathogenesis of ischemia/reperfusion-induced mucosal injury in mouse stomach. *Digestion,* 75, 188-97

Konturek, P. C., Brzozowski, T., Walter, B., Burnat, G., Hess, T., Hahn, E.G., and Konturek, S.J. (2006). Gherlin induced gastroprotective against ischemia reperfusion injuryinvolves an activation of sensory afferent nerves and hyperemia mediated by nitric oxide. *European Journal of Pharmacology*, 536, 171-181.

Kulkarni S.K., Gohel M.S., and Wakely C. (2011). The ulcerated Leg severity assessment score for prediction of venous leg ulcer. *Pharmacologyonline,* 3, 573-585.

Kundu A.D., Barik B.R., Mandal D.N., Dey A.K., and Banerji A.(1989). Zizybernalic acid, a pentacyclic triterpenoid of *Ziziphus jujube*. *Phytochemistry,* 28(11), 3155-3158.

Kwieciente , S., Brzozowski, T., and Konturek, S.J. (2002). Effect of reactive oxygen Species action on gastric mucosa in various models of mucosal injury. *Journal of Physiological Pharmacology*, 53, 761-773.

La Casa C., Villegas I., Alarcon de la Lastra C., Motilva V. and Martin Calero M. (2000). Evidence for protective and antioxidant properties of rutin, a natural flavones, against ethanol induced gastric lesions. *Journal of Ethnopharmacology*, 71, 45-53.

Laine L., Takeuchi K. and Tarnawski, A. (2008). Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology*, 135, 41-60.

Lima Z.P., Severi J.A., Pellizzon C.H., Brito ARMS, Sohs P.N., Caceres A., Giron L.M.,Vilegas W., Hiruma-Lima C.A. (2006). Can the aqueous decoction of mango flowers be used as antiulcer agent? *Journal of Ethnopharmacology*, 106, 29-37.

Longman R.J., Douthwaite J., Sylvester P.A., Poulsom R.,Corfield A.P. and Thomas M.G. (2000). Coordinated localization of mucins and trefoil peptides in the ulcer associated cell lineage and the gastrointestinal mucosa. *Gut*, 47, 792-800.

Magistretti M.J., Conti M., and Cristoni A. (1988). Antiulcer activity of an anthocyanidin from *Vaccinum myrtillus Arzneimittle Forschung*. *Drug Research*,6,283-287

Marieb, E. (2001). *Human anatomy and Physiology*. San Francisco: Benjamin Cummings, pp. 414.

Marotta F., Tajiri H., Safran P., Fesce E. and Ideo G. (1999).Ethanol related gastric mucosal damage: evidence of a free radical-mediated mechanism and beneficial effect of oral supplementation with bionormalizer, a novel natural antioxidant. *Digestion*, 60 (6), 538-543.

Maity P., Bindu S., and Choubey V. (2008). Lansoprazole protects and heals gastric mucosa from non-steroidal anti-inflammatory drug-induced gastrophy by inhibiting mitochondrial as well as Fas-mediated death pathways with concurrent induction of mucosal cell renewal. *Journal of Biological Chemistry*, 28, 14391-14401.

Morimoto M., Shimohara K., Oshima S. and Sukamoto T. (1991). Effects of the new antiulcer agent KB-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, as compared to those of teprenone and cimetidine. *Japanese Journal of Pharmacology*, 57, 495-505.

Mustapha S.K., Bolori M.T., Ajayi N.A., Nggada H.A., Pindiga U.H., Gashua W. and Khalil M.I.A. (2007). Endoscopic findings and the frequency of *Helicobacter pylori* among dyspeptic patients in north-eastern Nigeria. *Journal of Gastroenterology*, 6, 1-7.

Nagano Y., Matsui H.and Muramatsu M. (2005). Rebamipide significantly inhibit indomethacin-induced mitochondrial damage, lipid peroxidation, and apoptosis in gastric epithelial RGM-1 cells. *Digestive Diseases and Science*, 50 (1), S76-S83.

Nakatami N. (2000). Phenolic antioxidants from herbs and spices. *Biofactors.*13(Suppl 1-4), 141-146.

Nelson, R. F. (2005). *An introduction to behavioral endocrinology* (3rd ed.). Sunderland, Mass: Sinauer Associates, Pp. 100.

Nishida K., Ohta Y., Kobayashi T. and Ishiguro I. (1997). Involvement of the xanthine-xanthine oxidase system and neutrophiles in the development of acute gastric mucosal lesions in rats with water immersion restraint stress. *Digestion*, 58, 340-351.

Nyaberi M.O., Onyanga C.A., Mathooka F.M., Maina Z.M., Makobe M. and Mwaura F. (2010). Evaluation of phytochemical, antioxidant and antibacterial activity of edible fruit extracts of *Ziziphus abyssinica A. Rich*. *Journal of Animal and plant sciences*, 6, 623-629.

Odabasoglu, F., Cakir, A., Suleyman, H., Aslan, A., Bayr, Y., Halici, M., Kaza, C. (2006). Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. *Journal of Ethnopharmacology*, 103, 59-65.

Ott M., Gogvadze V., Orrenius S., and Zhivotovsky B. (2007). Mitochondria, oxidative stress and cell death. Apoptosis, 12, 913-922.

Organization for Economic Cooperation and Development (OECD) (2001). *OECD Guidelines for the testing of chemicals*. *Acute Oral Toxicity-Fixed dose Procedure*, pp. 425, 1-26.

Pandit S., Sur T.K., Jana U., Bhattacharyya D. and Debnath P.K. (2000). Antiulcer effectof Shankha bhasma in rats: a preliminary study. *Indian Journal of Pharmacology*, 32, 378-80.

Pawlowska A.M., Camangi F., Bader A. and Braca A. (2000). Flavonoids of *Ziziphus jujube* and *Ziziphus spina-christi (L)* Wild (Rhamnaceae) fruits. *Food Chemistry*, 112, 858-862.

Penissi A. and Piezzi R. (1999). Effect of dehydroleucodine on mucus production. A quantitative study. *Digestive Diseases and Sciences*, 44, 708-712.

Pohle T., Brzozowski T., Becker J.C., Vander Voort I.R., Markmann A. and Konturek S.J. (2001). Oxygen metabolites in aspirin induced gastric damage in humans. Gastroprotection by vitamin C. *Aliment Pharmacology and Therapeutics*, 15, 677-87.

Rajagopalan H. (2004). Inactivation of hCDC4 can cause chromosomal instability. *Indian Journal of Pharmacology*, 53, 431-438.

Rang, H.P. (2003). Pharmacology (5th ed.) Edinburgh: Churchill Livingstone, Pp. 234. ISBN 0-443-07145-4.

Rates, S.M.K. (2001). Plants as source of drugs. *Toxicology*, 39, 603-613.

Repetto, M.G. and Llessuy, S.F. (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcer. *Brazilian Journal of Medical Biological Research*, 35, 523-53.

Safwan A.K., Mohammed M.L.A and Juair I.A. (2011). Antiulcer activity of *Ficus religiosa* stem bark ethanolic extract in rats. *Journal of Medicinal Plants Research*, 5(3), 354-359.

Saranya P. and Geetha A. (2010). A biochemical study on the gastroprotective effect of hydroalcoholic extract of *Andrographis paniculata* in rats. *Gastroenterology*, 4, 356-76.

Sarfaraz, A., Ansari S.H. and Porchezhian E. (2002). Antifungal activity of alcoholic extracts of *Ziziphus vulgaris* and *Acacia concinna*. *Hamdard Medicus*. Bait al-Hikmah, Karachi,Pakistan, 14(15), 42-45.

Seyler R.W., Olson J.W., and Maier R.J. (2001). Superoxide dismutase-deficient mutants of *Helicobacter pylori* are hypersensitive to oxidative stress and defective in host colonization. *Immunology*, 69, 4034-4040.

Shiv K., Ganachari M.S. and Banappa Nagoor V.S. (2004). Anti-inflammatory activity of *Ziziphus jujube Lamk.* leaves extract in rats. *Journal of Natural Remedies*, 4, 183-185.

Shristi B., Neha J., Indu B., and Rajesh G. (2012). A Review on some Indian Medicinal Plants for Antiulcer Activity. *Journal of Science Research Chemistry*, 1, 6-9.

Simoes, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., and Mentz, L. A. (2003). *Farmacognosia da planta ao Medicamento, 5th edition* , Editora da UFRGS: Porto Alegre, Brasil, pp.424.

Somasundaram S., Hayllar H., Rafi S., Wrigglesworth J.M., Macpherson A.J., Bjarnason (1995). A review and hypothesis of the chemical basis of non steroidal anti-inflammatory drug-induced damage to the gastrointestinal tract. *Journal of Gastroenterology*, 30, 289-299.

Suerbaum, A. and Michetti P. (2005). *Helicobacter pylori* infection. *European Journal of Medicine*, 347, 1175.

Suzuki M., Miura S. and Mori M. (1994). Rebamipide, a novel antiulcer agent, Attenuates *Helicobacter pylori* induced gastric mucosal cell injury associated with neutrophil derived oxidants. *Gastroenterology*, 35, 1375-1378.

Takeuchi K, Niida H., Ohuchi T. and Okabe S. (1994).Antiulcerogenic action of ethanolic extract of the resin from *Virola surinamensis*. *Digestive Diseases and Sciences*, 39, 2536-2542.

Tanaka H., Kosaka N., Tomaru A., Shuto K., Oghihara T. and Sato N. (1989). Augmentation of the gastric mucosal defense mechanism induced by KW-5805, a novel antiulcer agent. *Gastroenterology*, 24, 170-173.

Tarnawski, A.S.(2005). Cellular and molecular mechanisms of gastrointestinal ulcer healing. *Digestive Diseases and Sciences*, 50, 24-33.

Toma, W., Trigo, J.R., Bensuaski de Paula, A.C., Souza Brito, A.R.M. (2004). Preventive activity of pyrrolidine alkaloids from *Senecio brasiliensis* (Asteraceae) on gastric and duodenal induced on mice and rats. *Journal of Ethnopharmacology*, 95, 345-351.

Tombola, F., Campello, S., De luca, L., Ruggiero, P., Del Giudice, G., Papini, E., Zoratti,M. (2003). Plant polyphenols inhibit VacA, a toxin secreted by the gastric pathogen *Helicobacter pylori*. *FEBS Letter*, 543, 184-189.

Tripathi K.D. (2001). *Essentials of Medical Pharmacology*. New Delhi: Jaypee Brothers Medical Publishers, pp. 52-53.

Tsutsumi, S., Tomisato W., Takano T., Rokutan K, Tsuchiya T. (2002).Gastric irritant induced apoptosis in guinea pig gastric mucosal cells in primary culture. *Biochemistry and Biophysics Acta, 1589:168-180.*

Ugwa E., Abubakar M.Y and Ashimi, A. (2011). Antidiarrheal effect of aqueous extract of *Ziziphus abyssinica* root in castor oil induced rats. *Nigerian Journal of Basic and Clinical Sciences*, 12(2), 67-73.

Vasconcelos, S.M.M., Pereira, E.C., Chaves, E.M.C., Lobata, R.F.G., Barbosa-Filho, J.M.,and Patrocinio, M.C.A.(2010). Pharmacologic study of *Amburana cearensis* and *Aniba genus*. *In Recent Progress in Medicinal Plants, volume 30: Drug Plant IV*, Singh, V.K.,Govil, J.N., Eds., Studium Press: Houston, TX, USA, Volume 30, pp. 51-64.

Vasconcelos, P.C.P, Kushima, H., Andreo, M., Hiruma-Lima, C.A., Vilegas, W., Takahira,R.K. and Pellizon, C.H. (2008). Studies of gastric mucosa regeneration and safety promoted by *Mouriri pusa* treatment in acetic acid ulcer model. *Journal of Ethnopharmacology*, 131, 431-441.

Wada, K., Kamaisaki, Y., Kentoro, N., Kishimoto, Y., Ashida, K., Itoh, T. (1997). Effect of plannotol on gastric injury induced by ischemia-reperfusion in rats. *Journal of Pharmaceutical Pharmacology*, 49, 903-907.

Wan G. H. (1993). Open repair of perforated peptic ulcer using Jing Wan Hong ointment. *Gastroenterology*, 28, 226-234.

Wang, J. J. (2000). Effect of anisodamine on the reserpine-induced gastric mucosal lesion in rats. *Zhongguo Bingli Shengli Zazhi*, 3, 237-242.

World Health Organization Statistical Information System WHO mortality database (2009). Retrieved from http://www.who.int/healthinfo/statistics/mortality.

Yanai, K. and Tashiro, M. (2007). The physiological and pathophysiological roles of neuronal histamine: an insight from positron emission tomography studies. *Pharmacology and Therapeutics,* 113(1), 1-15.

Yelken B., Dorman T. and Erkasap S. (1999). Clonidine pretreatment inhibits stress-induced gastric ulcer in rats. *Anesthetic Analogy*, 89, 159-162.

Yuan Y., and Hunt R.H. (2006). Treatment of non steroidal anti-inflammatory drug and *Helicobacter pylori* gastroduodenal ulcer and hypersecretory states. *In Therapy of digestive disorders, edition* 2,315-316(Eds Wolfe M.M.) London, U.K., Elsevier.

Zakaria Z.A., Abdul Hisam E.E., Roflee M.S., Norhafizah M., Somchit M.N., Teh L.K. and Salleh M.Z. (2011). *In vivo* antiulcer activity of the aqueous extract of *Bauhinia purpurea l*eaf. *Journal of Ethnopharmacology, 137, 1047-1054.*

Ziegler, J. and Facchini, P.J. (2008). Alkaloid biosynthesis: metabolism and trafficking. *Annual Review of Plant Biology*, 59, 735-769.

APPENDIX I

ACUTE TOXICITY CALCULATIONS

Stock Concentration = 3000mg/ml.

Dose = 3000mg/kg

$$\frac{\text{Dose} \times \text{Volume}}{1000} = \frac{3000 \times 2}{1000} = 6\text{g in 2mls of distilled water.}$$

3g of the extract dissolved in 1ml of water. = 3000mg/ml.

Administration

Weight of the rat 200g = 0.2kg.

$$V = \frac{\text{Dose X weight of rat (kg)}}{\text{Stock Concentration}}$$

$$V = \frac{3000 \times 0.2}{3000} = 0.2\text{ml.}$$

0.2 ml from the stock was prepared and administered.

The same procedure was used in calculation for the dose of all extracts and fractions to the rats.

Rat2 weight of 179g
Rat3 weight of 151.5g
Rat4 weight of 164g
Rat5 weight of 177g

APPENDIX 2

STATISTICAL CALCULATIONS USED

All statistical significance of the tests were determined by using ANOVA at ($p< 0.05$)

(i). Mean $\bar{x}$ =

(ii). Standard deviation (std) =

(iii). TSS = SRS- CF Where

$$EMS = \frac{ESS}{Edf}$$

$$Trtms = \frac{Trtss}{Trtdf}$$

$$Computed\ F = \frac{Trtms}{EMS}$$

$$SED = \frac{EMS}{R}$$

$$CV = \frac{EMS}{Grand\ mean}$$

$$LSD = \sqrt{\frac{2EMS}{R}} \times tedf$$

X = mean

TSS= Total Sum of Square

EMS= Error Mean Square

Trtms = Treatment mean square

SED = Standard Error of Mean

LSD = Least Significance Difference

CV = Coefficient of Variation

R = replication

n-1 = Degree of freedom

CF = Correction Factor

SRS = Sum of Replication Square.