

**EVALUATION OF THE ANTI-ULCER PROPERTIES OF THE STEM-BARK  
EXTRACTS OF *Khaya senegalensis* (Desr.) A. JUSS IN ALBINO RATS**

**BY**

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**JANUARY, 2017**

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EXTRACTS OF *Khaya senegalensis* (Desr.) A. JUSS IN ALBINO RATS**

**Ramatu UMAR, DVM (ABU) 2012  
(P13VTPP8004)**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE  
STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE AWARD OF A DEGREE OF MASTER OF  
SCIENCE IN VETERINARY PHARMACOLOGY**

**DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY,  
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**JANUARY, 2017**

## DECLARATION

I declare that the work reported in this dissertation, entitled “**EVALUATION OF THE ANTI-ULCER PROPERTIES OF THE STEM-BARK EXTRACTS OF *KHAYA SENEGALENSIS* (DESR.) A. JUSS IN ALBINO RATS**” has been carried out by me in the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The information derived from literature has been duly acknowledged in the text and in the list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Ramatu UMAR

Name of student

\_\_\_\_\_

Signature

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Date

## CERTIFICATION

This dissertation entitled “**EVALUATION OF THE ANTI-ULCER PROPERTIES OF THE STEM-BARK EXTRACTS OF *KHAYA SENEGALENSIS* (DESR.) A. JUSS IN ALBINO RATS**” by Ramatu UMAR meets the regulations governing the award of the degree of Master of Science in Veterinary Pharmacology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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## **DEDICATION**

This work is dedicated to my family members for their love and understanding over the years.

## **Acknowledgements**

All praises and glory be to Almighty Allah, who has given me the strength, provision and sound health to carry out this work.

My sincere appreciation and thanks goes to my able supervisors, Drs. M.M. Suleiman and H.G. Mika'il for their inputs, contribution, guidance and assistance through this study in spite of their tight schedule of work. I am grateful for that.

I must also acknowledge Drs. B. Mohammed, B. Habibu, M. Tauheed, Mr. D. Otie, Mal. Y. Abdulraheem, Mal. S. Abdulrazak, Mal. S. Musa and the entire staff of the Departments of Veterinary Pharmacology and Toxicology, and Physiology, for their contribution to the success of this work.

My gratitude also goes to my parents, my beloved husband and siblings for their encouragement, moral and financial support, may Allah reward you abundantly. Finally, I wish to sincerely express my appreciation to all my friends and well-wishers.

## Abstract

The aim of the study was to evaluate the antiulcer properties of the stem-bark extracts of *Khaya senegalensis* in albino rats. The fresh stem-bark of *K. senegalensis* was collected and made into powdered form using a mortar and pestle. The powdered form of the bark was cold extracted using methanol. The crude methanol extract (CME) was concentrated to dryness using rotary evaporator. The solid extract was further dissolved in distilled water and partitioned using *n*-hexane and ethyl acetate. The aqueous methanol (AMF), hexane (HEF) and ethyl acetate (EAF) fractions were also concentrated *in vacuo*. The CME, AMF, HEF and EAF were screened for the presence of phytochemical constituents using standard techniques. Similarly the AMF, HEF and EAF were evaluated for acute toxicity, gastric anti-secretory effects and gastroprotective effects using a rat-model. The stomach homogenates were assayed for levels of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA). Sample of the stomach tissues obtained from all treated rats were fixed in 10 % formalin and process for histopathological examination. The LD<sub>50</sub> was calculated to be >5000 mg/kg. Phytochemical analysis revealed the presence of carbohydrate, cardiac glycosides, saponins, flavonoids, steroids/triterpenes, alkaloids and tannins in the CME and AMF. The EAF contains all the metabolites except alkaloids while the HEF contains only steroids/triterpenes and flavonoids. The *n*-hexane (HEF), ethyl acetate (EAF) and the aqueous methanol (AMF) fractions did not produce any apparent toxic effect or mortality when tested at doses between 10 and 5000 mg/kg and the LD<sub>50</sub> was assumed to be ≥5000 mg/kg. The EAF decreases significantly ( $p < 0.05$ ) the concentration of gastric HCl

in rats when given at doses of 400 and 800 mg/kg. Similarly, the AMF significantly ( $p < 0.05$ ) decreased gastric HCl secretion at a dose of 800 mg/kg only. The ulcer indices of rats treated with the AMF, EAF and HEF at 200, 400, 800 mg/kg and misoprostol ( $5\mu\text{g/kg}$ ) were found to be significantly ( $p < 0.05$ ) lower when compared with the normal saline (untreated) control group. There was a significant ( $p < 0.05$ ) increase in the concentration of gastric mucus in rats treated with the AMF, EAF and HEF (200, 400 and 800 mg/kg) when compared with the normal saline (untreated) control group. The AMF and EAF significantly ( $p < 0.05$ ) reduced the concentration of malondialdehyde in the gastric tissue relative to rats treated with normal saline (5 ml/kg). There was a significant ( $p < 0.05$ ) increase in the SOD activity of the groups treated with AMF, EAF and HEF when compared with the normal saline (untreated) control group. There was a significant ( $p < 0.05$ ) decrease in the CAT activity of the groups treated with AMF and EAF when compared with the normal saline (untreated) control group. The stomach tissue was also processed for histopathological examinations. Histopathological changes were observed in the stomach of rats treated with AMF at the dose of 200 (necrosis and loss of epithelium lining) and 400 mg/kg (mild disruption on the surface epithelium), but no changes observed in the stomach of rats treated with AMF at the dose of 800 mg/kg (no disruption to the surface epithelium). The EAF treated groups at the dose of 200 (mild excoriation of the epithelium lining), 400 (loss of epithelium lining) and 800 mg/kg (mild loss of epithelium lining) showed milder lesions to the surface epithelium. Mild disruption to the surface epithelium were also observed in rats treated with HEF 800 mg/kg. The normal saline untreated group showed severe necrosis and loss of epithelium lining. In conclusion,

this study suggests that the extracts of *K. senegalensis* have anti-secretory, cytoprotective and antioxidant properties similar to that of a standard drug.

## Table of Contents

<b>CONTENTS</b>	<b>PAGE</b>
Cover page.....	i
Title page .....	iii
Declaration .....	iv
Certification .....	v
Dedication .....	vi
Acknowledgements .....	vii
Abstract .....	viii
Table of contents .....	xi
List of figures .....	xv
List of tables .....	xvi
List of plates.....	xvii
List of abbreviations.....	xix
<b>CHAPTER ONE: .....</b>	<b>1</b>
<b>1.0 INTRODUCTION.....</b>	<b>1</b>
<b>1.1 Statement of Research Problem.....</b>	<b>5</b>
<b>1.2 Justification of the Study.....</b>	<b>6</b>
<b>1.3 Aim of the Study .....</b>	<b>7</b>
<b>1.4 Objective of the Study .....</b>	<b>7</b>
<b>1.5 Null Hypothesis.....</b>	<b>8</b>
<b>CHAPTER TWO: .....</b>	<b>9</b>
<b>2.0 LITERATURE REVIEW.....</b>	<b>9</b>
<b>2.1 Peptic Ulcer.....</b>	<b>9</b>
<b>2.2 Pathophysiology of Peptic Ulcer .....</b>	<b>9</b>
<b>2.3 Signs and Symptoms.....</b>	<b>13</b>

<b>2.4 Classification of Peptic Ulcers .....</b>	<b>14</b>
<b>2.5 Chemotherapy of Peptic Ulcers.....</b>	<b>14</b>
<b>2.5.1 Proton pump inhibitors .....</b>	<b>16</b>
<b>2.5.2 H<sub>2</sub> blockers.....</b>	<b>17</b>
<b>2.5.3 Antibiotics.....</b>	<b>18</b>
<b>2.5.4 Bismuth salicylate.....</b>	<b>19</b>
<b>2.5.5 Prostaglandin E analogs .....</b>	<b>19</b>
<b>2.5.6 Antacids.....</b>	<b>20</b>
<b>2.6 Medicinal Plants with Anti-ulcer Properties.....</b>	<b>20</b>
<b>2.7 <i>Khaya senegalensis</i>.....</b>	<b>22</b>
<b>2.8 Nomenclature.....</b>	<b>23</b>
<b>2.8.1 Common names.....</b>	<b>23</b>
<b>2.9 Traditional Medicinal Uses.....</b>	<b>23</b>
<b>2.10 Bioactive Metabolites.....</b>	<b>24</b>
<b>CHAPTER THREE: .....</b>	<b>27</b>
<b>3.0 MATERIALS AND METHODS.....</b>	<b>27</b>
<b>3.1 Location of the Experiment.....</b>	<b>27</b>
<b>3.2 Plant Collection, Identification and Preparation .....</b>	<b>27</b>
<b>3.3 Plant Extraction.....</b>	<b>27</b>
<b>3.4 Fractionation.....</b>	<b>28</b>
<b>3.5 Phytochemical Screening of the Extracts.....</b>	<b>28</b>
<b>3.5.1 Test for carbohydrate.....</b>	<b>28</b>
<b>3.5.1.1 Molish's test.....</b>	<b>28</b>
<b>3.5.1.2 Fehling's solution test.....</b>	<b>29</b>
<b>3.5.2 Test for saponins (frothing test).....</b>	<b>29</b>
<b>3.5.3 Test for alkaloids.....</b>	<b>29</b>
<b>3.5.3.1 Dragendoff's test.....</b>	<b>29</b>
<b>3.5.3.2 Mayer's test.....</b>	<b>29</b>
<b>3.5.4 Test for tannins.....</b>	<b>30</b>
<b>3.5.4.1 Lead sub-acetate test.....</b>	<b>30</b>
<b>3.5.4.2 Ferric chloride test.....</b>	<b>30</b>
<b>3.5.5 Test for flavonoids.....</b>	<b>30</b>

3.5.5.1 <i>Shinoda test</i> .....	30
3.5.5.2 <i>Sodium hydroxide test</i> .....	30
<b>3.5.6 Test for cardiac glycosides</b> .....	<b>31</b>
3.5.6.1 <i>Kelle-killiani test</i> .....	31
3.5.6.2 <i>Salkowski's test</i> .....	31
<b>3.5.7 Test for anthraquinones</b> .....	<b>31</b>
3.5.7.1 <i>Test for free anthraquinones (Bontrager's test)</i> .....	31
3.5.7.2 <i>Test for combined anthracene (modified Bontrager's test)</i> .....	31
<b>3.5.8 Test for steroids and triterpenes (Lieberman-Buchard test)</b> .....	<b>32</b>
<b>3.6 Experimental Animals</b> .....	<b>32</b>
<b>3.7 Experimental Design</b> .....	<b>32</b>
<b>3.7.1 Acute toxicity studies</b> .....	<b>32</b>
<b>3.7.2 Experimental anti-secretory studies</b> .....	<b>33</b>
<b>3.7.3 Measurement of gastric acid</b> .....	<b>34</b>
<b>3.7.4 Gastroprotective activity</b> .....	<b>34</b>
<b>3.7.5 Gastric mucus determination</b> .....	<b>35</b>
<b>3.7.6 Gastric malondialdehyde determination</b> .....	<b>35</b>
<b>3.7.7 Catalase activity (CAT)</b> .....	<b>36</b>
<b>3.7.8 Superoxide dismutase (SOD)</b> .....	<b>36</b>
<b>3.8 Histopathology</b> .....	<b>37</b>
<b>3.9 Statistical Analysis</b> .....	<b>37</b>
<b>CHAPTER FOUR: RESULTS</b> .....	<b>38</b>
<b>4.1 Plant Yield</b> .....	<b>38</b>
<b>4.2 Phytochemical Screening of the Plant Extracts</b> .....	<b>38</b>
<b>4.3 Acute Toxicity Studies</b> .....	<b>40</b>
<b>4.4 Effect of Fractions on Gastric Acid</b> .....	<b>40</b>

<b>4.5 Effect of Fractions on Gastric Fluid.....</b>	<b>42</b>
<b>4.6 Effect of Fractions on Ethanol-induced Gastric Ulcer.....</b>	<b>44</b>
<b>4.7 Effect of Fractions on Gastric Mucus Production.....</b>	<b>46</b>
<b>4.8 Effect of Fractions on Malondialdehyde.....</b>	<b>48</b>
<b>4.9 Effect of Fractions on Stomach Antioxidant Enzymes.....</b>	<b>50</b>
<b>4.9.1 Effect of Fractions on Stomach Superoxide Dismutase Activity.....</b>	<b>50</b>
<b>4.9.2 Effect of Fractions on Catalase Activity.....</b>	<b>52</b>
<b>4.10 Effect of Fractions on Stomach Histo-architecture.....</b>	<b>54</b>
<b>CHAPTER FIVE: DISCUSSION.....</b>	<b>64</b>
<b>CHAPTER SIX: .....</b>	<b>70</b>
<b>6.0 CONCLUSION AND RECOMMENDATIONS.....</b>	<b>70</b>
<b>6.1 Conclusion.....</b>	<b>70</b>
<b>6.2 Recommendations.....</b>	<b>71</b>
<b>REFERENCES.....</b>	<b>72</b>
<b>APPENDICES.....</b>	<b>84</b>

**LIST OF FIGURES.....xiv**

Figure 4.1 The effect of the fractions of *Khaya senegalensis* and cimetidine on gastric acid secretion in rats..... 41

Figure 4.2 The effect of the fractions of *Khaya senegalensis* and cimetidine on gastric fluid in rats..... 43

Figure 4.3 The ulcer protective effect of the fractions of *Khaya senegalensis* and misoprostol against ethanol-induced gastric ulceration in rats..... 45

Figure 4.4 The effect of the fractions of *Khaya senegalensis* and misoprostol on gastric mucus production in rats..... 47

Figure 4.5 The effect of the fractions of *Khaya senegalensis* and misoprostol on gastric malondialdehyde concentration in rats..... 49

Figure 4.6 The effect of the fractions of *Khaya senegalensis* and misoprostol on stomach superoxide dismutase activity in rats..... 51

Figure 4.7 The effect of the fractions of *Khaya senegalensis* and misoprostol on stomach catalase activity in rats..... 53

**LIST OF TABLES.....xv**  
Table 4.1 Phytochemical screening of the stem-bark of *Khaya senegalensis*.....39

<b>LIST OF PLATES.....</b>	<b>xvi</b>
Plate 1: <i>Khaya senegalensis</i> in its natural habitat.....	26
Plate 2: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with aqueous methanol fraction of <i>Khaya senegalensis</i> (200 mg/kg) showing necrosis and loss of epithelium lining (H and E ×100).....	55
Plate 3: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with aqueous methanol fraction of <i>Khaya senegalensis</i> (400 mg/kg) showing mild disruption on the surface epithelium (H and E ×100).....	56
Plate 4: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with aqueous methanol fraction of <i>Khaya senegalensis</i> (800 mg/kg) showing no disruption to the surface epithelium (H and E ×100).....	57
Plate 5: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with ethyl acetate fraction of <i>Khaya senegalensis</i> (200 mg/kg) showing mild excoriation of the epithelium lining (H and E × 100).....	58
Plate 6: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with ethyl acetate fraction of <i>Khaya senegalensis</i> (400 mg/kg) showing loss of epithelium lining (H and E × 100).....	59
Plate 7: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with ethyl acetate fraction of <i>Khaya senegalensis</i> (800 mg/kg) showing mild loss of epithelium lining (H and E × 100).....	60

Plate 8: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with hexane fraction of *Khaya senegalensis* (800 mg/kg) showing mild disruption to the surface epithelium (H and E × 100).....61

Plate 9: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with Misoprostol (5 µg/kg) showing mild excoriation to the surface epithelium (H and E × 100).....62

Plate 10: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80 % ethanol and treated with normal saline (5 ml/kg) showing severe necrosis and loss of epithelium lining (H and E × 100).....63

## LIST OF ABBREVIATIONS

ABU	Ahmadu Bello University	
AMF	Aqueous methanol fraction	
CAT	Catalase	
CME	Crude methanol extract	
CogA	Cytotoxin-associated gene A	
COX	Cyclooxygenase	
EAF	Ethylacetate fraction	
HCl	Hydrochloric acid	
HEF	Hexane fraction	
LD <sub>50</sub>	Median lethal dose	
MDA	Malondialdehyde	
NO	Nitric oxide	
NSAIDs	Non-steroidal anti-inflammatory drugs	
PAF	Platelet activating factor	
ROS	Reactive oxygen species	
SOD	Superoxide dismutase	
TBA	Thiobarbituric acid	
TCA	Trichloroacetic acid	
TNF	Tumor necrosis factor	
XO	Xanthine	oxidase

## CHAPTER ONE

### 1.0

### INTRODUCTION

Peptic ulcer is defined as a break off in the continuity of the mucosa of stomach (gastric ulcer) or few centimeters of duodenum (duodenal ulcer) (Verma, 2010). Basically, the word “peptic” is derived from Greek term “peptikos” whose meaning is related to digestion. Peptic ulcer is a gastro-intestinal disorder due to an imbalance between the aggressive factors like acid, pepsin, non-steroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* infection and defensive factors like bicarbonate ion secretion, prostaglandins, gastric mucus, and innate resistance of the mucosal cell factors (Dashputre and Naikwade, 2011). Peptic ulcer usually develops when aggressive factors overcome the defensive factors (Izzo and Borrelli, 2000). The major factors that disrupt the equilibrium of the integrity of the stomach mucosa are *Helicobacter pylori*, acid-pepsin hyper secretion, non-steroidal anti-inflammatory drugs, sometimes idiopathic due to usage of tobacco, psychological stress, rapid gastric emptying and Zollinger-Ellison syndrome where there is a high and uncontrollable production of acid also leads to ulcer formation (Baron and Calam, 2001; Nayaka *et al.*, 2011). Several reports showed that old age is a factor that contribute to formation of gastric ulcer. Older people are more sensitive to the ulcer causing effects of NSAIDs. Younger individuals have higher risk of duodenal ulcers (Lunevicius and Morkevicius, 2005; Pahwa *et al.*, 2010).

The pathogenesis of peptic ulcer disease includes a complex imbalance between gastric offensive factors like acid, pepsin secretion, *Helicobacter pylori* bacterium, bile salts, ethanol, some medications like the non-steroidal anti-inflammatory drugs(NSAIDs), lipid peroxidation, nitric oxide (NO) and defensive mucosal factors; prostaglandins, gastric mucus, cellular renovation, blood flow, mucosal cell shedding, glycoproteins, mucin secretion, enterocyte proliferation and

antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD) and glutathione) (Marietta and John, 2010; Tripathy and Afrin, 2016). Peptic ulcer can be categorized on the basis of location and on the severity of disease. Numerous other factors are also responsible for progression of peptic ulcers like tumor necrosis factor- $\alpha$  (TNF  $\alpha$ ), reactive oxygen species (ROS), release of histamine, incidence of apoptosis and bile acids secretion (Singha *et al.*, 2008; Fatemeh *et al.*, 2011).

The NSAIDs-induced ulcers are very common, NSAIDs helps in progression of ulcer by inhibiting the enzyme cyclooxygenase (COX 2). COX 2 converts arachidonic acid into prostaglandins, which protects the stomach mucosal barrier. Inhibition of COX 2 could result in corrosive action to the stomach mucosa with pepsin, HCl and other corrosive substances cause the progression of peptic ulcers. These drugs are able not only to exert gastric injuring effects, but also to delay the healing of ulcer lesions through a variety of local and systemic mechanisms (Musumba *et al.*, 2009; Wolfe *et al.*, 1999).

Since the discovery that prostaglandin biosynthesis could be inhibited by NSAIDs through the blockade of cyclooxygenase (COX) enzymes, there has been a great interest in the contribution of prostaglandins to the mechanisms of gastric mucosal defense. Gastric injury associated with NSAIDs administration depends partly on cyclooxygenase inhibition and partly on cyclooxygenase-independent mechanisms, which result mainly from local direct actions (Scarpignato and Hunt, 2010). Cyclooxygenase blockade has been shown to increase the susceptibility of gastric mucosa to NSAID-induced injury by suppression of a number of prostaglandin-mediated protective functions. It has also been recognized that NSAIDs can interfere with gastric mucosal physiology by causing alterations unrelated to the inhibition of

prostaglandin biosynthesis, such as oxidative stress and changes in epithelial cell proliferation/apoptosis balance (Davies and Wallace, 1997).

Most NSAIDs are weakly acidic in nature and this property accounts for their local cyclooxygenase-independent injuring actions on the gastric mucosa. In the presence of gastric acid, the undissociated lipophilic form of acidic NSAIDs can impair the hydrophobic surface barrier of the stomach. Currently, it is acknowledged that NSAIDs can impair gastric protection through the blockade of COX-1 (Wallace, 2006).

There is a documented evidence that showed oxygen derived free radical role in the pathogenesis of the injury of various tissues, including the digestive system. The role of TNF- $\alpha$  in the pathogenesis of gastric ulcer is well documented. TNF- $\alpha$  is a cytokine which initiates the inflammatory process through stimulation of migration of leucocytes into inflammatory sites therefore plays a significant role in formation of gastric ulcers (Lychkova *et al.*, 2010). Moreover *H. pylori* is also implicated in peptic ulcer progression. *H. pylori* is a gram negative bacillus, motile, microaerophilic, flagellated, spiral shaped bacterium, which secretes the distinct enzyme urease that converts urea to ammonia, which further reduces the acidity of stomach (Pandey *et al.*, 2010). Type I strains of *H. pylori* possess a pathogenic activity, that encodes the effector protein cytotoxin-associated gene A (cagA). After translocation into the host cell, cagA affects cell shape, increases cell motility, disturbs cell junctional activity and thus responsible for gastric carcinomas and gastric ulcers (Buti *et al.*, 2011).

Drugs that are currently used to treat peptic ulcers include proton pump inhibitors (omeprazole, lansoprazole), H<sub>2</sub> receptors antagonist (cimetidine, ranitidine), cytoprotectants (misoprostol), demulcents (aluminium hydroxide, methycellulose), anticholinergics (methantheline), antacids (kaolin, magnesium and aluminium salts). However, these drugs are used but with some level of

toxicity. H<sub>2</sub> receptor antagonists have been shown to cause impotency in men. The proton pump inhibitors is an unforeseeable cause for hypergastrinemia and atrophic gastritis. The use of antacids leads to stomach distention, belching, constipation and there is risk of ulcer perforation and other drugs like anticholinergics induce constipation, dry mouth, urinary retention, blurred vision, xerostomia and precipitation of glaucoma (Reilly, 1999; Franko and Richter, 2002). Cytoprotectants cause constipation, trigger diarrhoea, dizziness, oedema and hypophosphatemia whereas abdominal cramps, uterine bleeding and abortion is the probable cause of prostaglandin analogues (Akthar *et al.*, 1992).

Herbal medicine is fast emerging treatment as an alternative to available synthetic drugs for treatment of ulcer, possibly due to lower costs and less side effects. Many tropical herbs have been scientifically reported to possess potent anti-ulcer activity (Vela *et al.*, 1997; Singh *et al.*, 2008). Various chemical compounds have been isolated from medicinal plants with anti-ulcer activity (Lewis and Hanson, 1991; Dharmani *et al.*, 2004).

*Khaya senegalensis* is a deciduous evergreen tree that grows up to 15-30 m high and up to 3.0 m in diameter. The plant is often recognized by its round deciduous evergreen crown of dark shining foliage, round capsules, reddish-tinged scales, pinnate leaves and short bole covered with dark grey bark, woody capsule and bitter yielding gum when wounded. The flowers have pale green sepals, creamy petals, stamina tube and bright-red disc beneath the ovary and bark conspicuous panicles. Leaves have 3 to 4 (sometimes 5) pair of leaflets and are about 5.0-10 cm long, 2.5-5.0 cm broad with a more or less elliptic, round, obtuse or very acuminate at the apex. The wood is typically reddish-brown mahogany (Keay, 1989). Its dark and heavy timber, which is said to provide the best surface-finishing of all the African mahoganies, is a popular timber for lorry bodies, construction work, and decking in boats, in addition to its traditional uses for

furniture (Styles and White, 1991). It grows primarily in the deciduous savannah forests, and is found mainly in West Africa as well as countries such as Sudan, Uganda, and Democratic Republic of Congo.

The plant is commonly called African mahogany, Khaya wood, Gambia mahogany or Heavy African mahogany in English. In Nigeria, the plant is known as *madaci*, *ogonwa*, *ono* and *dalchi* or *kahi* in Hausa, Yoruba, Igbo and Fulani languages, respectively.

### **1.1 Statement of Research Problem**

Peptic ulcers have become one of the major human and animal illness throughout the world, affecting nearly 8–10% of the global population (Baron and Calam, 2001), and of these number, 5% suffer from gastric ulcers (Bandyopadhyay *et al.*, 2001). Diverse factors such as alcohol consumption, a stressful lifestyle, use of non-steroidal anti-inflammatory drugs (NSAIDs) and drugs which stimulate gastric acid and pepsin secretion, *Helicobacter pylori* infections and smoking contribute to the pathogenesis of gastric ulcers (Rang *et al.*, 2012).

Approximately 11-14% of men and 8-11% of women will develop peptic ulcer disease in their life time. The mortality due to peptic ulcer disease is approximately one death per 10,000 cases. Peptic ulcer can occur at any age, although they are rare in children and teenagers. Peptic ulcer causes serious complication such as bleeding. The most common sign of peptic ulcer is abdominal pain, while other symptoms include nausea, vomiting, loss of appetite and loss of weight (Bandyopadhyay *et al.*, 2001).

Gastric ulcers occur in both young and adult animals, but higher percentage of ulcer is known to occur in matured animals. Likewise animals used in sport such as dogs do suffer more from gastric ulceration compared to other canine species. Endoscopic studies have revealed gastric

ulceration in 48.5% of dogs used in sport. Incidence of gastric ulcers ranging from 25-50% have been reported in foals (Bao, 2010). However, higher incidence of gastric ulcers (60%) have been reported in matured horses (Bao, 2010). The incidence of gastric ulceration in relation to the exciting cause also varies significantly from species to species. Ulcers have been reported in dogs, cats, rodents and horses secondary to administration of a variety of NSAIDs (Grainek *et al.*, 2008).

## **1.2 Justification of the Study**

Herbal medicines are considered as better alternatives for the treatment of peptic ulcer because the currently used drugs produce several side effects (Vanisree *et al.*, 2002). The proton pump inhibitors (omeprazole, lansoprazole) may cause nausea, abdominal pain, constipation and diarrhoea. The proton pump inhibitors are also an unforeseeable cause for hypergastrinemia and atrophic gastritis. The H<sub>2</sub> receptor antagonists (cimetidine, ranitidine) may cause gynaecomastia, loss of libido and impotency. The use of antacids leads to stomach distention, belching, constipation and there is risk of ulcer perforation and other drugs like anticholinergics induce constipation, dry mouth, urinary retention, blurred vision, xerostomia and precipitation of glaucoma (Reilly, 1999; Franko and Richter, 1998). Cytoprotectives cause constipation, trigger diarrhoea, dizziness, oedema and hypophosphatemia whereas abdominal cramps, uterine bleeding and abortion is the probable cause of prostaglandin analogues (Akthar *et al.*, 1992).

Due to the occurrence of many side effects by use of synthetic drugs for many diseases, medicinal plants are considered as the main source of new drugs as they have fewer or no side effects. As herbal medicines are considered as safe for the treatment of ulcers with lesser adverse effects, economical, effective and relatively less toxic.

The extract of *Khaya senegalensis* used in the traditional treatment of gastric ulcers (Dalziel, 1956). The crude methanol extract of the stem-bark of *Khaya senegalensis* has been shown to possess anti-ulcer activity against ethanol induced gastric ulceration in rats (Suleiman *et al.*, 2013).

Research is still ongoing in the search for potent anti-ulcer agents of plant origin (Srivastava *et al.*, 2011, Vinay *et al.*, 2005). The development of alternative anti-ulcer drugs that are effective and cheaper is therefore very important. Several medicinal plant species like *Ocimum sanctum*, *Carica papaya*, *Desmodium gangeticum*, *Allophylus serratus*, *Hemidesmus indicus* and *Asparagus racemosus* have been reported to possess anti-ulcer activity (Lakshmi *et al.*, 2013).

In addition, about 80 % of the world's population relies on traditional medicinal plants for treating many diseases including gastric ulcers. (Marini-Bettolo, 1980). Commercial cultivation of medicinal plants will help generate income for the youths, reduce unemployment and provide revenue for the country.

### **1.3 Aim of the Study**

The aim of this study was to evaluate the anti-ulcer properties of the stem-bark extracts of *Khaya senegalensis* in albino rats.

### **1.4 Objectives of the Study**

The objectives of the study were to investigate the:

- i. phytochemical constituents present in *K. senegalensis* stem-bark extracts.
- ii. acute toxicity effect of extracts of *K. senegalensis* in rats by determining its median lethal dose (LD<sub>50</sub>).
- iii. effect of extracts of *K. senegalensis* on experimentally-induced gastric ulcer in rats.

- iv. antioxidant effect of *K. senegalensis* extract in rats experimentally-induced with gastric ulcers.
- v. cytoprotective effect of *K. senegalensis* in rats experimentally-induced with gastric ulceration.
- vi. effects of the stem-bark extracts of *Khaya senegalensis* on gastric acid (HCl) secretion in rats.

## **1.5**

### **Null Hypothesis**

The extracts of the stem-bark of *Khaya senegalensis* do not have anti-ulcer and antioxidant properties in rats.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Peptic Ulcer

Peptic ulcer is a condition in which there is a discontinuity in the entire thickness of gastric or duodenal mucosa that persists as a result of acid and pepsin in gastric juice (Vinay *et al.*, 2014). Peptic ulcer can also be describe as an erosion in the lining of the stomach or duodenum which is caused by the disruptions of the gastric mucosal defense and repair systems (Ubaka *et al.*, 2010). Such factors could range from natural causes (gastric cancer), infections (*H. pylori*), and lifestyle (drugs like non-steroidal anti- inflammatory agents, alcohol, stress and cigarette smoking) (Anoop and Jegadeesan, 2003).

#### 2.2 Pathophysiology of Peptic Ulcer

Peptic ulcers are open sores that develop on the inside mucosal lining of the digestive tract, specifically, the initial portion of the small intestine (duodenum), gastric portion of the oesophagus and stomach. Peptic ulcers develop when the balance between the digestive acids and the protective mucosal layer is disrupted. In healthy animals, the digestive tract is coated with a mucous membrane that protects the underlying tissue against the highly corrosive digestive acid; however, if the amount of acid is increased, or the pH of the acid is significantly reduced, or the mucous membrane layer becomes too thin or dry, the acid damages the tissue and ulceration ensues (Dufton, 2012).

Gastric ulcer is a type of peptic ulcer that affects the stomach lining due to an imbalance between gastric acid and the gastric mucosa. The most common type of peptic ulcer is not gastric ulcer, but rather duodenal ulcer. Duodenal ulcers are more diagnosed each year compared to gastric ulcers (Dufton, 2012). The reasons for these are not completely understood, but may be related to

the tissue of the stomach lining being more resilient to oxidation by strong acids compared to the tissue of the duodenum.

For many decades, peptic ulcer disease was thought to be primarily a result of emotional stress and diets rich in spicy foods. However, in the mid-1980s, *Helicobacter pylori* bacteria was discovered in the stomach of many patients with gastritis and peptic ulceration and recognized to be a significant causative agent (Marshall and Warren, 1984). Recent studies estimate that at least 70 percent, and possibly as high as 90 percent, of gastric ulcers are caused by *H. pylori* colonization, which results in antral mucosa erosion of the stomach, chronic inflammation, and ulcer formation (Huang *et al.*, 2002).

*Helicobacter pylori* are corkscrew-shaped bacteria that commonly live and multiply within the mucous layer that covers and protects tissue lining the stomach and duodenum. The presence of *H. pylori* is usually benign and leads to no damage or negative consequences within the gastrointestinal tract. However, due to factors that are not entirely understood, *H. pylori* sometimes overgrows and is unable to be contained by the immune system despite the appearance of antibodies (Dufton, 2012). The bacteria penetrate and disrupt the mucous layer and inflame the stomach lining, leading to chronic inflammation or gastritis. Chronic gastritis results in an inability to regulate gastrin, which is a peptide hormone that stimulates secretion of gastric acid (hydrochloric acid) by the parietal cells of the stomach and aids in gastric motility. Reduced regulation sometimes results in an increase of gastrin secretion, but in most cases, production is decreased, which results in either hypochlorhydria (low stomach acidity) or achlorhydria (no stomach acid).

*H. pylori* infections that lead to increased gastrin production can erode the mucosal lining of the stomach and incite ulcer formation (Vadokas *et al.*, 1997). On the other hand, chronically low

production of gastrin leads to reduced digestive ability and its related sequelae, such as malabsorption, leaky gut syndrome, inflammatory reactions, and various gastrointestinal symptoms. It is not clear if transmitting *H. pylori* from person to person via saliva is a significant risk factor in developing gastric ulcers, or if a biochemical change in the bacteria's host environment is the only causal factor. The flora and fauna of every healthy gastrointestinal system includes *H. pylori* and numerous other types of bacteria, which normally coexist with the host symbiotically.

Another major cause of gastric ulceration is the regular use of non-steroidal anti-inflammatory drugs (NSAIDs), especially those that are classified as COX-1 inhibitors (Martin *et al.*, 2008). The growth and maintenance of stomach mucus is stimulated by certain prostaglandins, which are blocked by most NSAIDs. Specifically, COX-1 inhibitors, such as aspirin, ibuprofen, naproxen and ketoprofen, block the function of cyclooxygenase-1, which is essential for the production of prostaglandins needed to make the gastric mucosal lining. COX-2 selective NSAIDs, such as celecoxib preferentially inhibit cyclooxygenase-2, which is less essential for the growth of gastric mucosa. As such, use of COX-2 inhibitors represent roughly half the risk of NSAID-related gastric ulceration when compared to COX-1 inhibitors (Lanza *et al.*, 2009). It has been predicted that as the prevalence of *H. pylori*-caused ulceration declines in industrialized countries due to more appropriate medical treatment, a greater proportion of gastric ulcers will be due to increasing NSAID use among people with pain syndromes and arthritic conditions (Bashinskaya *et al.*, 2011).

Even though it has been discovered and confirmed that bacterial infection plays a dominant role in the development of gastric and other peptic ulcers, emotional stress is still considered a significant causative factor (Wachirawat *et al.*, 2003). Most researchers agree that ulcers are not

purely an infectious disease and that psychological factors do play an important role, although emotional stress is highly subjective and very difficult to quantify. Stress might promote *H. pylori* infection and ulceration by causing an excess production of stomach acid (Kim *et al.*, 2002).

Other causes of gastric ulcers include conditions that can result in direct damage to the wall of the stomach lining such as alcohol consumption, radiation therapy, burns, and physical trauma to the abdomen. In addition to NSAIDs, over-use or misuse of virtually any oral pharmaceuticals can also precipitate gastric inflammation and ulceration, especially those medications used to treat osteoporosis (bisphosphonates examples Actonel, Fosamax, others) (Malfertheiner *et al.*, 2009). Pathogenesis of gastric ulcer also involves oxidative stress with antioxidants playing a very important role in order to protect gastric mucosa (Trivedi and Rawal, 2001) and repairing gastric damage.

Risk factors that have been identified for gastric ulcers include tobacco smoking and chewing, moderate-to-high levels of alcohol consumption, moderate-to-high levels of coffee consumption, high-stress occupations, severe emotional trauma, long-term use of pharmaceutical medications, chronic pain, surgical procedures, advancing age, and heredity (Salih *et al.*, 2007).

In terms of appearance, gastric ulcers are usually round or oval, between 2 and 4 cm in diameter, and located on the lesser curvature of the stomach. Gastric ulcers penetrate the muscularis mucosae and muscularis propria layers of the stomach by acid-pepsin aggression. The ulcer is usually smooth with regular and perpendicular borders. In contrast, irregular borders are often a sign of ulceration due to stomach cancer. During the active phase of gastric ulceration, the base of the ulcer consists of four zones: inflammatory exudate, fibrinoid necrosis, granulation tissue

and fibrous tissue (Paul *et al.*, 2008). The fibrous base of the ulcer may contain vessels with thickened walls or display thrombosis.

### **2.3 Signs and Symptoms**

Burning-type abdominal pain is the most common symptom of peptic ulcers. With gastric ulcers, the pain is felt predominantly in the middle of the abdomen, although it can be felt from the navel to the sternum and clavicles, and posteriorly in the thoracic region of the spine (Malfertheiner *et al.*, 2009). The pain is caused by the tissue ulceration, which exposes nerve fibers, and aggravated by gastric acid coming in contact with the ulcerated area. The pain is typically worse with an empty stomach and about three hours after meals, although it may flare-up after eating spicy and acidic foods. Conversely, the burning pain may be temporarily relieved by eating certain foods or drinking beverages that buffer stomach acid, such as milk. The pain from gastric ulcers is rarely constant; rather, it fluctuates during the day, often flares up during the night, and might disappear for many days and return again (Dufton, 2012).

Gastric ulcers often bleed and produce distinctive signs and symptoms. Signs of bleeding in the upper digestive tract, including the stomach, are different from the signs from the large intestine and bowel. Bleeding from gastric ulcers usually lead to black or tarry stool and vomit that looks like coffee grounds (if the blood is old and partially digested) or colored bright red (if the blood is fresh) (Fauci, 2008). Tarry stool from internal bleeding, known as melena, is foul-smelling due to the oxidized iron of the hemoglobin. In contrast, bleeding from ulceration in the lower gastrointestinal tract usually produces bright red blood in the faeces, but no blood in the vomitus. Vomiting is not a typical symptom of lower gastrointestinal bleeding (Cortese *et al.*, 2010).

In addition to intense pain and vomiting, other signs and symptoms of gastric ulcers include loss of appetite, unexplained weight loss, heartburn, indigestion, belching, bloating and nausea.

Peptic ulcers if left untreated can result in anemia from loss of blood, infection (peritonitis) from tissue perforation, and scar tissue that can block passage of food through the digestive tract. Sudden, excessive bleeding can be life-threatening, especially if the ulcer erodes one of the blood vessels, such as the gastroduodenal artery (Banerjee *et al.*, 2010). Stomach cancer is between three and six times more likely to develop from gastric ulceration caused by *H. pylori* infection (Malfertheiner *et al.*, 2009). On the other hand, about four percent of stomach ulcers are actually caused by a malignant tumor, sometimes stomach biopsies are needed to rule-out cancer (Fauci, 2008).

#### **2.4 Classification of Peptic Ulcers**

Classification of peptic ulcers is very important as the treatment regimens varies widely for each class of peptic ulcers. The ulcers are classified according to their depth in the gastrointestinal mucosa, anatomical location in the gastrointestinal area and the severity of the clinical presentation. Gastroduodenal ulcers may be glandular mucosal ulceration, generalized gastritis, focal duodenal ulceration, duodenal erosions or duodenitis (Parrah *et al.*, 2013). Depending upon the depth of the ulcer, ulcers can be superficial or deep and range from simple epithelial erosion to full thickness bleeding or perforating ulcers. And on the basis of clinical presentation, ulcers may be acute or chronic (Bao, 2010).

#### **2.5 Chemotherapy of Peptic Ulcers**

Systemic acid reducing drugs fall into the categories of histamine ( $H_2$ )-blocking agents (Cimetidine, ranitidine, famotidine) and proton pump inhibitors (omeprazole, lansoprazole, rabeprazole).  $H_2$  blocking agents reduce hydrochloric acid production that is stimulated at histamine receptors. Proton pump inhibitor decrease hydrochloric acid production from oxyntic cells by inhibiting the  $H^+/K^+$ -ATPase and work independently of specific receptors and thus

may be more effective. Besides this acid reducing drugs, are those drugsthat act on cellular metabolism to inhibit hydrogen ion secretion (e.g. prostaglandins such as misoprostol). A Cytoprotectedrug such as sucralfate is important for protecting ulcerated tissue (Parrah *et al.*, 2013).The modern history of peptic ulcer disease has followed a remarkable course and lead to widespread acceptance of the notion that the majority of gastric ulcers are caused by *H. pylori* and the institution of treatment protocols such as “triple therapy,” which has had dramatic effects (greater than 90 percent initial eradication rate and less than 10 percent re-infection rate at five years) (Qasim and O’marain, 2002). Triple therapy is the use of a proton-pump inhibitor or H<sub>2</sub> blocker (which both reduce gastric acid secretion) with either two different antibiotics or an antibiotic combined with bismuth salicylate. The discovery of effective and potent acid suppressants and the identification of the largely bacteria cause of peptic ulceration are the two most important developments that are associated with the overall decreased rates seen with peptic ulcer disease (Malfertheiner *et al.*, 2009). Additionally, the morbidity associated with *H. pylori* infection has dramatically decreased from 1993 to present due mainly to triple therapy (Bashinskaya *et al.*, 2011).

Although the overall rates of peptic ulcer disease have decreased, incidence of gastric ulceration has increased over the last few decades due to increased NSAID use. Thus, antibiotics or triple therapy are not given to patients with gastric ulcers caused by NSAIDs; rather, their pain medications are either reduced, eliminated or altered, and acid-reducing medications may be recommended for a few months to allow the ulceration to heal.

### **2.5.1 Proton-pump inhibitors**

Proton-pump inhibitors are a group of drugs that display pronounced and long-lasting reduction of stomach acid production. They are among the most widely sold drugs in the world and are generally considered the most potent inhibitors of acid secretion, more so than an earlier class of inhibitors called H<sub>2</sub>-receptor antagonists (or simply H<sub>2</sub> blockers) (Dufton, 2012). Proton-pump inhibitors act by irreversibly blocking the hydrogen / potassium adenosine triphosphatase enzyme system (commonly called the gastric proton pump) of the stomach's parietal cells. The proton-pump is the final stage in stomach acid secretion, being directly responsible for secreting hydrogen ions into the gastric lumen and making it an ideal target for inhibiting acid secretion. Proton-pump inhibitors reduce gastric acid secretion by up to 99 percent. Proton-pump inhibitors are given in an inactive form, which readily crosses cell membranes and enters into parietal cells, where they become activated by the acidic environment.

The lack of stomach acid aids in the healing of gastric ulcers and reduces the pain from indigestion and heartburn, but hydrochloric acid is required for the digestion of proteins and absorption of nutrients, especially vitamin B-12 and calcium. As such, hypochlorhydria (insufficient hydrochloric acid) can lead to a variety of side effects such as B-12 deficiency (which often mimics symptoms of Alzheimer's), increased risk of bone fracture, increased risk of certain heart arrhythmias and interstitial nephritis, low serum magnesium levels (hypomagnesemia), headaches (the most commonly reported adverse effect), nausea, diarrhoea, abdominal pain, flatulence, constipation, fatigue and dizziness (Qasim and O'marain, 2002). Furthermore, recent data suggests that there may be a rebound effect when proton-pump inhibitors are discontinued (Reimer *et al.*, 2009). Ceasation on the use of proton-pump can cause an increase in stomach acid production above that of normal levels that lasts for several weeks.

The most common clinically used proton-pump inhibitors include omeprazole, lansoprazole, dexlansoprazole, esomeprazole, pantoprazole and rabeprazole. The vast majority of these drugs are known as benzimidazole derivatives, but new research indicates that imidazopyridine derivatives may be a more effective means of treatment (Sachs *et al.*, 2006).

### **2.5.2 H<sub>2</sub> blockers**

H<sub>2</sub> blockers, also known as histamine blockers or H<sub>2</sub>-receptor antagonists, are competitive antagonists of histamine at the parietal cell H<sub>2</sub>-receptors in the stomach. H<sub>2</sub> blockers suppress the secretion of hydrochloric acid from parietal cells by two mechanisms: histamine released by enterochromaffin-like cells in the stomach is blocked from binding on parietal cell H<sub>2</sub>-receptors, which stimulate acid secretion; and as a consequence, other substances that promote acid secretion (such as gastrin and acetylcholine) have a reduced effect on parietal cells when the H<sub>2</sub>-receptors are blocked. H<sub>2</sub> blockers are still commonly used for the treatment of dyspepsia, but they have been surpassed in popularity by the more effective proton-pump inhibitors for the treatment of gastric ulcers (Parrah *et al.*, 2013).

The approved members of the H<sub>2</sub> blocker group are cimetidine, ranitidine, famotidine and nizatidine. They are available over-the-counter in relatively low doses, or by prescription in larger doses. H<sub>2</sub> blockers are generally well-tolerated like proton-pump inhibitors, with the exception of cimetidine, which was the first H<sub>2</sub> blocker developed in the mid-to-late 1960s. Cimetidine became the first “blockbuster drug”, but it was discovered to interfere with the body's mechanisms of drug metabolism and elimination through the liver cytochrome P450 pathway. As such, cimetidine use increases the risk of drug toxicity, and has also been linked to hypotension, gynecomastia in males, loss of libido, and impotence (Reilly, 1999; Franko and Richter, 2002). Ranitidine was introduced in 1981 and was found to have a far better tolerability profile (fewer

adverse drug reactions), longer-lasting action, and ten-times the biochemical activity of cimetidine. However, the most common side effects of H<sub>2</sub> blockers can be similar to most proton-pump inhibitors because they too greatly reduce the production of hydrochloric acid, which is needed for healthy digestion and the absorption of vitamin B-12 and calcium (Vanisree *et al.*, 2002). Due to their adverse effects on digestion and nutrient absorption, acid-reducing medications are only recommended for a consecutive duration of about two months.

### **2.5.3 Antibiotics**

Antibiotics are prescribed to kill bacteria such as *H. pylori*, but due to resistance and adaptability, more than one type is often recommended to be taken at the same time. Antibiotic regimens are different throughout the world, antibiotics prescribed for treating *H. pylori* include amoxicillin, clarithromycin (Biaxin), metronidazole (Flagyl), furazolidone (Furoxone) and tetracycline. Antibiotics are usually prescribed for only two weeks at a time in order to avoid side effects. Relatively common side effects of antibiotic use include acquired resistance to antibiotic therapy, serious allergic reactions (including anaphylaxis), nausea, stomach upset, diarrhea, sun sensitivity, disruption of the intestinal flora and fauna, systemic overgrowth of pathogenic bacteria (such as *Clostridium difficile*) and yeast species (such as *Candida albicans*), and numerous potential interactions with other drugs (Dufton, 2012).

Clarithromycin-based triple therapy (combined with a proton-pump inhibitor and either amoxicillin or metronidazole for 10-14 days) is considered the standard treatment for an ulcer caused by *H. pylori*; however, other protocols are also popular. Research shows higher cure rates with 14 days of treatment, although side effects become much more probable and severe beyond this time frame (Dufton, 2012). Another strategy, called “salvage” or quadruple therapy, involves adding bismuth salicylate compounds to the treatment protocol.

#### **2.5.4 Bismuth salicylate**

Medications that protect the lining of the stomach and small intestine from the damaging effects of acid are called cytoprotective agents which include sucralfate (Carafate) and over-the-counter agents such as bismuth salicylate (Pepto-Bismol). Bismuth compounds may also directly kill *H. pylori*, although its antibacterial actions should not be viewed as a replacement for conventional antibiotics. Bismuth salicylate, like all salicylates (especially aspirin), can cause serious bleeding problems when used alone in patients with bleeding ulcers. Bismuth quadruple therapy usually involves combining bismuth salicylate with a proton-pump inhibitor, tetracycline and metronidazole for 10-14 days. The main reasons to add bismuth salicylate to a treatment regime for gastric ulcers is if the patient is still infected with *H. pylori* after a trial of triple therapy, or if the patient cannot take amoxicillin (a penicillin-like antibiotic) because of a penicillin allergy, or if the patient has been treated before with a macrolide antibiotic, such as clarithromycin. Side effects from bismuth salicylate are considered rare, but the most common are benign and include darkening of the stools and/or tongue, and a metallic taste in the mouth (Danesh *et al.*, 1998).

#### **2.5.5 Prostaglandin E analogues**

Prostaglandin analogues (e.g. misoprostol) may improve ulcer healing or prevent ulcer formation by increasing mucosal circulation, vascular permeability or mucosal cell turnover and migration. The drugs inhibit adenylate cyclase, reducing cyclic AMP production and thereby reducing the protein kinase activity essential to hydrogen ion generation. It is the drug of choice for NSAID-induced ulceration and so more effective than sucralfate or H<sub>2</sub> receptor antagonists in preventing NSAID-induced ulceration (Lanza *et al.*, 2009). Some patients develop diarrhoea, which is usually self-limiting. Prostaglandin E<sub>2</sub> is not used in hypotensive animals because gastric damage may be increased.

### **2.5.6 Antacids**

Antacids are available over-the-counter. They neutralize existing stomach acid, which can provide relief of burning stomach pain, heartburn, and indigestion, but they are not considered as a treatment for gastric ulcers. Antacids do not kill *H. pylori* nor do they block the production of stomach acid. Commonly used antacids include aluminum hydroxide, magnesium hydroxide (Milk of Magnesia), aluminum hydroxide combined with magnesium hydroxide, calcium carbonate, and sodium bicarbonate. It is recommended to take antacids at least one hour before or two hours after taking other medications because antacids may block the medications from being absorbed and effectively utilized. Other side effects can include constipation or diarrhea, depending on the main ingredients of the antacids (Washabau and Halt, 1995).

### **2.6 Medicinal Plants with Anti-ulcer Properties**

*Allophylus serratus Kurz* is commonly known as Tippani. It belongs to the family Sapindaceae and used to treat elephantiasis, oedema, and inflammation, fracture of bones, dyspepsia, anorexia and diarrhoea. Phytochemical studies proved the presence of phenacetamide, betasitosterol, and quercetin. The mechanism involved in production of antiulcer activity by plant leaf extract is cytoprotective mechanism as well as anti-secretory (Vinay *et al.*, 2005). The ethanolic leaf extract of the plant proved to possess antibacterial activity (Gaikwad and Chavan, 2013). The plant is reported to possess anti osteoporotic activity (Naibedyet *al.*, 2010).

*Aloe vera* (L.) Burm.f. is commonly known as Aloe. It belongs to the family Xanthorrhoeaceae. Reported constituents in plant are aminoacids, anthraquinones, enzymes, hormones, lignin, minerals, salicylic acid, saponins, sterols, sugars, vitamins (Sharma and Shilpi, 2011). The anti-ulcer activity of the plant is reported in Indomethacin induced ulcer model. The mechanism involved in production of anti-ulcer activity of the plant is due to its antioxidant, anti-

inflammatory, mucus secreting, cytoprotective or healing activities (Sai *et al.*, 2011). Reported pharmacological activities of the plant are hypoglycemic, hypolipidemic, woundhealing, immunomodulatory, antifungal and hepatoprotective effects (Justin and Joseph, 2010; Bhriagu *et al.*, 2016).

*Desmostachya bipinnata* (L.) Stapf is commonly known as Saved gram belongs to the family Gramineae. Kaempferol, quercetin, quercetin-3-glucoside, trycin and trycin-7-glucoside were isolated from the ethanolic extract of plant. The ethanolic extract of aerial parts of the plant reported anti-ulcer activity (Awaad *et al.*, 2008). The methanolic extract revealed anti helicobacter activity at MIC value of 40 µg/ml (Mohammed and Safwat, 2009). The alcoholic and aqueous root extracts reported anti- diarrhoeal activity in rats against castor oil induced diarrhoea and charcoal meal test (Ashoket *et al.*, 2010). The methanolic root extract of the plant exerted *in vitro* anticancer activity (Rajasekaranet *et al.*, 2012).

*Capsicum annuum* L. is commonly known as Chilli pepper and it is most widely cultivated throughout the world. It belongs to the family Solanaceae. The fruit is proved to possess anti-ulcer activity, antioxidant activity (Asad *et al.*, 2011). The methanolic seed extract of the plant reported antiobesity activity in 3T3-L1 adipocyte (Geonuket *et al.*, 2010). The fruit and vegetable peel extracts of the plant exerted radical- scavenging properties. Solasonine present in the plant reported platelet aggregation inhibitory activity (Guang-Yanet *et al.*, 2008).

*Carica papaya* Linn. is commonly known as Papaya. It belongs to the family Caricaceae and well known for various medicinal properties. The fruits are reported to possess anti-ulcer activity (Ologunduduet *et al.*, 2008). The seeds are reported to exert antimicrobial, anthelmintic, antiamoebic properties. The fruit had shown hepatoprotective activity and also used for pediatrics burns .The seed extracts are proved to exhibit pronounced hypertrophy and

hyperplasia of pituitary gonadotrophs. The pollen from flowers of the plant possess histaminergic properties (Dumppierret *et al.*, 1998; Tripathy and Afrin 2016).

*Ocimum sanctum* Linn. is commonly known as Holy basil belonging to the family Labiatae and widely used for prevention and cure of many illnesses and everyday ailments. The plant fixed oil reported anti-ulcer activity due to its lipoxigenase inhibitory, histamine antagonistic and antisecretory effects. The plant is reported for anticancer activity against human fibro sarcoma cells culture. Oral administration of the plant leaf extract was reported for hypoglycemic effect in normal, glucose fed hyperglycemic and streptozotocin-induced diabetic rats (Lakshmi *et al.*, 2013). Reported pharmacological activities of the plant are anti-bacterial, anti-inflammatory, antihypertensive, cardioprotective, central nervous system depressant, antioxidant, chemopreventive, immunomodulatory, analgesic, anti-pyretic, anti-fertility, anti-arthritic, anti-stress, anti-cataract, anticoagulant, hepatoprotective and radioprotective effects (Govind and Madhuri, 2010).

## 2.7

### *Khaya senegalensis*

*Khaya senegalensis* (Ders.) A. Juss is a large and sturdy tree (up to 35m high with a diameter of 1 to 3m) of Meliaceae family. Also named Senegal mahogany, it is a forestry species well known and exploited by Africans (Belem *et al.*, 2008). It has pinnate leaves, glabrous with 6 to 12 alternate or opposite elliptical-oblong leaflets. At the flowering, *K. senegalensis* twigs carry at their ends panicles of small white flowers consisting of successive whorls of four floral parts. Its fruits are capsules with thick and woody seed coat. These capsules are dehiscent and have four valves that allow to see the flat seeds closely applied against each other. The bark of this tree is very thick, scaly and dark brownish-gray colour. In section it oozes reddish exudates.

*Khaya senegalensis* favourable habitats are those in wet soils, deep; alluvial; the edge of streams and non-flooded lowlands. It also accommodates dry or superficial or lateritic stations when rainfall is between 650-1300 mm during 4-7 months. It inhabits Sudanese and Sudanese-Guinean regions (Sokpon and Ouinsavi, 2002). It is abundant in the woodlands of most countries in the Guinea Gulf (Nigeria, Cameroon, Gabon, Benin, Togo, Ivory Coast, Guinea, Guinea Bissau, Gambia and Senegal) where it is known under various local names and where its usefulness is widely reported (Djouecheet *al.*, 2011).

## **2.8 Nomenclature**

*K. senegalensis* belongs to the Kingdom plantae, Division magnoliophyta, Class magnoliopsida, Order sapindales, Family meliaceae, Genus *Khaya*, species *senegalensis*. The binomial name therefore is *Khaya senegalensis* (Belemet *al.*, 2008).

### **2.8.1 Common names**

The plant is commonly called African mahogany, dry zone mahogany, Gambia mahogany, khaya wood, or Senegal mahogany in English; homraya, homra, murraya, or mahogoni in Arabic; cailcedrat, Acajou d'Afrique, or acajou du Sénégal in French. In Nigeria, the plant is known as madaci, ono, and ogonwo in Hausa, Igbo and Yoruba languages, respectively (Abubakar *et al.*, 2009).

## **2.9 Traditional Medicinal Uses**

*K. senegalensis* is one of the most popular medicinal Meliaceae plants used in traditional African remedies. The decoction from the stem-bark is commonly used as a bitter tonic in folk and popular medicines for malaria, fever, mucous diarrhoea, and venereal diseases as well as for an anthelmintic against taeniasis (Iwu, 1993; Olayinka *et al.*, 1992). The plant extracts have been reported to exhibit anti-inflammatory effects (Lompoet *al.*, 1998) as well as anti-bacterial

(Koné *et al.*, 2004), anthelmintic (Ademola *et al.*, 2004), anti-tumor, anti-oxidant (Androulakis *et al.*, 2006), and anti-plasmodial activities (El-Tahir *et al.*, 1998). An extract of the bark is used for treating dermatoses, scorpion stings, jaundice, infection of the gum and constipation. The flowers are used to treat stomach diseases and as an ingredient in anti-syphilitic prescription. A decoction of the leaves is used in Ghana to maturate small pox pustules (Dalziel, 1956). According to Nacoulma (1996), the aqueous extract is taken against gynaecological disturbances, digestive disorders and nervous confusion. The crude methanol extract of the stem-bark of the plant has been shown to possess anti-ulcer activity against ethanol induced gastric ulceration in rats (Suleiman *et al.*, 2013).

## **2.10 Bioactive metabolites**

There was an extensive research on the chemical constituents of the stem-bark extract of *K. senegalensis* for the past four decades (Adesida *et al.*, 1971; Mulholland *et al.*, 2000; Narender *et al.*, 2007).

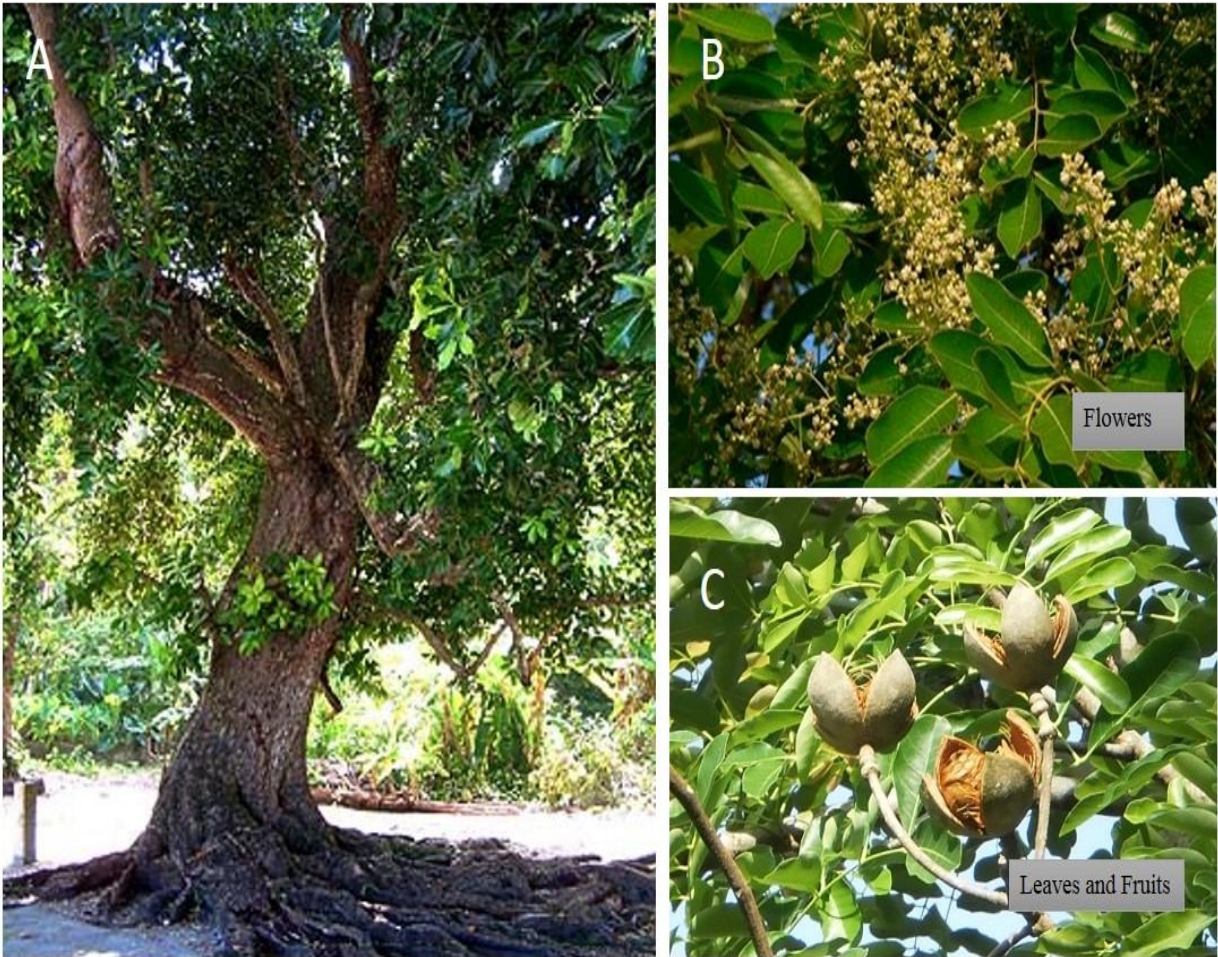
Two dimeric flavonoids (bis [3, 4-dihydroxy-7,8-furano-5,6-monomethylallyl-oxy]-5-C-5-biflavonyl and [4-hydroxy-7-methyl-3-C- $\alpha$ -L-rhamnopyranosyl]-5-C-5-[4-hydroxy-7-methyl-3-C- $\alpha$ -D-glucopyranosyl] biflavonoid.), as well as 2,6-dihydroxy-p-benzoquinone,  $\beta$ -sistosterol, and 3-O-glucose- $\beta$ -sistosterol, catechin, tannins, saponins, polysaccharides and coumarins (Kayser and Abreu 2001), with immuno-stimulating activity have been isolated from the stem-bark extract of the plant. Approximately 45 different limonoids (Adesida *et al.*, 1971; Olmo *et al.*, 1996; Govindachari *et al.*, 1998; Mulholland *et al.*, 2000; Abdelgaleil *et al.*, 2003; Tchimine *et al.*, 2006) were isolated from the plant leaf, seed, and stem-bark, which constitute the bitter part of the plant. Furthermore, some limonoids isolated have exhibited feeding deterrent and growth inhibitory properties (El-Aswad *et al.*, 2003), anti-feeding (Abdelgaleil *et al.*, 2003), antifungal

(Govindachari *et al.*, 1998; Abdelgaleil *et al.*, 2004), and anti-sickling activities (Fallet *et al.*, 1999).

Flavonoids were reported to exhibit antioxidant (Tapas *et al.*, 2008; Ferreira *et al.*, 2010) and anti-inflammatory (Sandhar *et al.*, 2011) activities. Flavonoids are able to activate the mucosal defense system through stimulation of gastric mucus secretion and scavenge for the ROS and free radicals produced by ethanol (Abdelwahab *et al.*, 2011).

Flavonoids are able to decrease ulcerogenic lesions by promoting the formation of gastric mucosa, inhibit the production of pepsinogen and diminish acid mucosal secretion (La Casa *et al.*, 2000). Kelly *et al.*, (2009) reported that flavonoids have antihistaminic properties, thus, decreases histamine levels, as well as preventing the release of histamine from gastric mast cells, and inhibiting the gastric  $H^+/K^+$  proton pump and diminishing gastric acid secretion. Mota *et al.* (2009) have summarized the literature on 95 flavonoids with varying degrees of antiulcerogenic activity, confirming that flavonoids have a therapeutic potential for the more effective treatment of peptic ulcers.

Alkaloids such as atropine and scopolamine constitute an important class of anticholinergic compounds and clinically, they are used to block the muscarinic activity of acetylcholine thereby showing antisecretory effects in the treatment of peptic ulcer (Maria *et al.*, 2008). On the other hand, tannins are known to protect the outer most layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation (Asuzu and Onu, 1990). Saponins may exert its protective activities in ulceration by the activation of mucous membrane protective factors (Choudhary *et al.*, 2013).



**Plate 1: *Khaya senegalensis* in its natural habitat; (A) whole plant, (B) flowers and (C) leaves and fruits**

### **CHAPTER THREE**

## **3.0 MATERIALS AND METHODS**

### **3.1 Location of the Experiment**

The phytochemical screening of the extracts of *K. senegalensis* was done in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, while the Pharmacological evaluation was carried out in the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University (A.B.U), Zaria.

### **3.2 Plant Collection, Identification and Preparation**

The stem-bark of *K. senegalensis* was collected around the main campus of Ahmadu Bello University, Zaria, Nigeria. The leaves and seeds samples of the plant were sent to the Herbarium, Department of Biological Sciences, A.B.U, Zaria, for identification. A voucher specimen number 900181 was deposited at the Herbarium for reference purpose. The stem-bark was dried in the laboratory at room temperature, and the dried plant part was made into powdered form using wooden mortar and pestle.

### **3.3 Plant Extraction**

Two kilogrammes of the powdered stem-bark was weighed and macerated with 6 litres of methanol for 48 hours and filtered. Thereafter, the filtrate obtained was concentrated on a water bath at 40 °C.

### **3.4 Fractionation**

Two hundred and thirty grams of the crude methanol extract was dissolved in 1 litre of distilled water. The solution was transferred to a separating funnel and 500 ml of *n*-hexane was added. The funnel was agitated gently and then allowed to stand undisturbed. The tap was then gently opened and the lower denser aqueous portion was collected into a conical flask. The remaining upper portion was dispensed into a clean conical flask to obtain the *n*-hexane fraction. The procedure was repeated twice and the *n*-hexane fractions were pooled together. The aqueous fraction was transferred to the separating funnel again and 500ml of ethylacetate was added in a similar way and fractionated in the same fashion. The remaining aqueous fraction was concentrated on a water bath at 40 °C (Harbone, 1994).

### **3.5 Phytochemical Screening of the Extracts**

Phytochemical screening of the fractions of *K. senegalensis* stem-bark was carried out to detect the presence of metabolites (carbohydrates, anthraquinones, flavonoids, tannins, alkaloid, saponins, cardiac glycosides, steroids and triterpenes) present in the plant using standard method (Silva *et al.*, 1998).

#### **3.5.1 Test for carbohydrates**

##### *3.5.1.1 Molish's test*

Half gram of the extract was dissolved in 3 ml of water and later few drops of Molish's reagent in concentrated sulphuric acid added to the mixture. The appearance of a purple colour at the interface is indicative of carbohydrate in the extract.

##### *3.5.1.2 Fehling's test*

Half gram of the extract was dissolved in 2 ml of water and to the mixture; 5 ml of Fehling's solutions A and B in the ratio of 1:1 was added and boiled for 5 minutes. The appearance of a brick-red precipitate is indicative of free reducing sugar.

### **3.5.2 Test for Saponins (frothing test)**

Two hundred milligrams of the extract was dissolved with distilled water in a test tube. The mixture was shaken vigorously for 30 seconds and was allowed to stand for 20 minutes. A honey comb-like structure that foamed for 30 minutes indicates the presence of saponins in the extract.

### **3.5.3 Test for alkaloids**

#### *3.5.3.1 Dragendoff's test*

Half gram of the extract was taken in a test tube and dissolved in distilled water. Few drops of Dragendoff's reagent were added to the extract. The appearance of a precipitate indicates the presence of an alkaloid in the extract.

#### *3.5.3.2 Mayer's test*

Half gram of the extract was taken and dissolved in a test tube. Few drops of Meyer's reagent were added to the extract. Formation of a precipitate indicated the presence of alkaloids.

### **3.5.4 Test for tannins**

#### *3.5.4.1 Lead sub-acetate test*

Half gram of the extract was added to the test tube and dissolved in 3 ml of distilled water; this was followed by addition of 3 drops of lead sub-acetate solution. The appearance of a dark-green precipitate indicates the presence of tannins.

#### *3.5.4.2 Ferric chloride test*

Half gram of the extract was added to the test tube and dissolved in 10 ml of distilled water and filtered. Few drops of ferric chloride solution were then added to the filtrate. The formation of a blue-black precipitate indicates the presence of hydrolysable tannins while the appearance of a green precipitate indicates the presence of condensed tannin in the extract.

### **3.5.5 Test for flavonoids**

#### *3.5.5.1 Shinoda test*

Half gram of the extract was taken and dissolved in a test tube using about 2 ml of 50 % methanol and the mixture was heated. Few chips of magnesium and 4 to 5 drops of concentrated hydrochloric acid were added to the mixture. An orange colouration indicates the presence of flavonoids aglycones in the extract.

#### *3.5.5.2 Sodium hydroxide test*

Half gram of the extract was added to the test tube and dissolved in 5 ml of distilled water. Few drops of aqueous sodium hydroxide were added to the extract. A yellow colouration indicates the presence of flavonoid aglycones in the extract.

### **3.5.6 Test for cardiac glycosides**

#### *3.5.6.1 Kelle-Killiani test*

Half gram of the extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45 degrees and 1 ml of concentrated sulphuric acid was

added at the side of the tube. A purple ring at the interface indicates the presence of cardiac glycosides.

#### *3.5.6.2 Salkowski test*

Half gram of the extract was dissolved in 2 ml of chloroform and to the solution; few drops of concentrated sulphuric acid were added to form a lower layer. A reddish-brown colour at the chloroform-acid interface indicates the presence of steroidal ring.

### **3.5.7 Test for anthraquinones**

#### *3.5.7.1 Bontragers test*

One gram of the extract was taken in a test tube and 5 ml of benzene in equal volume of water was added and shaken, thereafter, 5 ml of 10 % of ammonia solution was added to the filtrate and stirred. Absence of colour change indicates the absence of free anthraquinones.

#### *3.5.7.2 Modified Bontragers test*

One gram of the extract was added to the test tube and dissolved in 5 ml of distilled water. The solution was then boiled with 5 ml of 10 % sulphuric acid for 3 minutes. This process hydrolyses the glycosides to give rise to the aglycones which are soluble in hot water only. The solution was filtered hot and filtrate was cooled and extracted with 5 ml of benzene. The benzene layer was filtered off and shaken gently with half its volume of 10 % ammonia solution. No colour change observed in the mixture indicates the absence of anthracene compounds.

### **3.5.8 Test for steroids and triterpenes (Lieberman-Buchard test)**

Half gram of the extract was taken and dissolved in a test tube and dissolved in 1 ml of acetic anhydride was added to the extract. In addition, 1 ml of concentrated sulphuric acid was carefully added to the mixture along the side of the test tube. The immediate appearance of

purple colour indicates the presence of triterpenes while the late appearance of a blue or blue-green colouration in the test tube is indicative of steroids.

### **3.6 Experimental Animals**

Rats of both sex weighing between 150 and 180 g were obtained from Animal House, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were conditioned to the laboratory environment in plastic cages for 2 weeks before the commencement of the experiment. All animals were fed with groundnut cake and growers mash in the ratio of 1:4 and water was provided *ad-libitum*. All animal experimentation were done according to the Ahmadu Bello University Animal Use and Care Guidelines.

### **3.7 Experimental Design**

#### **3.7.1 Acute toxicity studies**

The method of Lorke (1983) was used to determine the median lethal dose (LD<sub>50</sub>) of the extracts in rats. In all the studies, the extracts were dissolved in distilled water before administration to the animals. In the first part of the trial, 9 albino rats were randomly allocated into 3 groups of 3 rats each. The rats were deprived of food for 24 hours and water for 12 hours. Groups 1, 2 and 3 were treated with the extract orally at 10, 100 and 1000 mg/kg body weight, respectively. Rats were observed for 48 hours for any sign of toxicity or mortality. The second part of the trial was done based on the results obtained during the initial trial. In the second part of the experiment, 3 rats were assigned into 3 groups of 1 rat each. Rats in groups 1, 2 and 3 were given the extract at 1600, 2900 and 5000 mg/kg, respectively. All animals were observed as earlier described.

#### **3.7.2 Experimental anti-secretory studies**

Twenty five rats were randomly allocated into 5 groups of 5 rats each. The animals were fasted for food 24 hours prior to surgery but not water. All rats were anaesthetized using ketamine HCl at 20 mg/kg given by intramuscular route. A ventral midline incision was made on each rat and the stomach exteriorized and ligated at the pyloric region. Groups 1, 2 and 3 were treated with the aqueous fraction at 200, 400, and 800 mg/kg respectively, while groups 4 and 5 were given cimetidine at 20 mg/kg and normal saline at 5 ml/kg, respectively. The treatments were given intra-gastrically. After treatment, the stomach in each rat was returned into the abdominal cavity and the incisions sutured. Animals were allowed to recover from anaesthesia. Four hours after recovery from anaesthesia, all animals were euthanized in a chloroform chamber. The sutured area of individual animals were opened again, the stomach removed and its contents were emptied into sample container and measured. Thereafter, the content of each stomach was centrifuged at 3000 g for 5 minutes and the supernatant was decanted (Telesphore *et al.*, 2005). Similarly, the *n*-hexane and ethyl acetate fractions of *K. senegalensis* obtained during partitioning were evaluated for anti-secretory effect in the same fashion.

### **3.7.3 Measurement of gastric acid**

The supernatant collected was titrated with 0.01 M sodium hydroxide (NaOH) to end point using 2 % phenolphthalein as an indicator. Total acidity was calculated and was expressed as mEq/ml in each sample (Telesphore *et al.*, 2005).

### **3.7.4 Gastroprotective activity**

Twenty five rats were randomly allocated into 5 groups of 5 rats each and were fasted for 24 hours for food but not water. Groups 1, 2 and 3 were treated with the aqueous fraction at 200, 400 and 800 mg/kg, respectively, while groups 4 and 5 were given misoprostol at 5 µg/kg and normal saline at 5 ml/kg, respectively. All treatments were administered orally. Thirty minutes after treatment with the extract, misoprostol and normal saline, ulcer was induced using 1ml of 80 % ethanol. All animals were sacrificed after three hours in chloroform chamber. The stomachs were removed and opened along the lesser curvature, rinsed, laid out on a flat surface, and examined for the presence of mucosal lesions. A ×2 hand lens was used to locate and score the lesions (Ohara *et al.*, 1995). Severity of the gastric mucosal damage was graded as follows: grade 0, no lesion; grade 1, haemorrhagic erosions (less than five); grade 2, haemorrhagic erosions (more than five); grade 3, many small linear ulcers (shorter than 2 mm) or single linear ulcer of marked size (larger than 2 mm); grade 4, multiple linear ulcer of marked size. The ulcer index for each group was calculated by multiplying the number of rats in each grade by the number of grade divided by the number of rats in each group (Yusuf *et al.*, 2004). Similarly, then-hexane and the ethyl acetate fractions of *K. senegalensis* obtained during partitioning were evaluated for gastroprotective activity in the same fashion, while still maintaining the control groups.

### **3.7.5 Gastric mucus determination**

Gastric mucus determination was done by the method described by Corne *et al.* (1974). Five hundred milligrams of the glandular portion of the stomach of each rat in the gastroprotective study was transferred immediately into 1 % Alcian blue solution (in 0.1 M sucrose solution, buffered with 0.05 M sodium acetate, pH 5.8); the excess dye was removed by rinsing in sucrose solution. The dye complexed with the gastric wall mucus was extracted with 0.5 M magnesium

chloride and 4 ml sample of the blue extract was shaken with an equal volume of diethyl ether and the resulting emulsion was centrifuged at 3000 g for 10 minutes. The supernatant was decanted and the absorbance was recorded at 560 nm. The quantity of Alcian blue extracted/g of the glandular tissue was calculated.

### **3.7.6 Gastric malondialdehyde determination**

Determination of malondialdehyde (MDA) concentration as an index of lipid peroxidation was done using the double method of Draper and Hadley (1990) modified by Yavuz *et al.*, (2004). The principle of the method is based on the spectrophotometric measurement of the colour developed during reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). Five hundred milligrams of the glandular portion of stomach from each animal was weighed and homogenized in ice-cold phosphate buffer to obtain a 10 % homogenate. Trichloroacetic acid (TCA) solution (2.5ml of 100g/L) was added to 0.5 ml stomach homogenate in a centrifuge tube, placed in boiling water bath for 15 minutes and cooled under tap water for another 5 minutes. The mixture was centrifuge at 1000 g for 10 minutes and 2 ml of the supernatant was added to 1 ml of 6.7 g/L of thiobarbituric acid (TBA) solution in a test tube. The mixture was boiled in water bath (100 °C) for 15 minutes, the solution was cooled under tap water and the absorbance was measured at 532 nm using spectrophotometer.

### **3.7.7 Catalase activity (CAT)**

Catalase activity was measured using Abebi's method (1974). Exactly 10 µl of tissue homogenate was added to a test tube containing 2.80ml of 50 mM potassium phosphate buffer (pH 7.0). The reaction initiated by adding 0.1ml of freshly prepared 30mM H<sub>2</sub>O<sub>2</sub> and the decomposition rate of H<sub>2</sub>O<sub>2</sub> was measured at 240nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of 0.041mM<sup>-1</sup>cm<sup>-1</sup> was used to calculate the catalase activity.

$$\text{Catalase Concentration} = \text{Absorbance}/E$$

$$\text{Catalase Activity} = \text{Catalase concentration}/\text{protein concentration (mg/ml)}$$

### **3.7.8 Superoxide dismutase (SOD)**

Superoxide dismutase was determined by the method described by Fridovich (1989). The principle of the method is the ability of superoxide dismutase to inhibit auto oxidation of adrenaline at pH 10.2. About 114.3g of Na<sub>2</sub>CO<sub>3</sub> and 4.2g of NaHCO<sub>3</sub> was dissolved in distilled water and made up to 100ml in a volumetric flask (buffered with 0.05M carbonate, pH 10.2). Ten milligrams of 0.3mM adrenaline was dissolved in 17ml of distilled water, the solution was prepared fresh. Tissue homogenate of 0.1ml was diluted in 0.9ml 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 0.3 ml 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. The absorbance was measured over 30 seconds up to 150 seconds at 480 nm.

$$\text{Increase in absorbance per minute} = (A_2 - A_1)/2.5$$

$$\% \text{ Inhibition} = 100 - [(\text{increase in absorbance for sample}/\text{increase in absorbance of blank}) \times 100]$$

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adrenochrome in 1 minute.

### **3.8 Histopathology**

Tissue sample obtained from the stomach of rats after the gastroprotective testing were preserved in 10% formalin and processed for histological examination as described by Luna (1960). Lesions observed were recorded and photographed.

### **3.9 Statistical Analysis**

Data obtained was expressed as mean  $\pm$  standard error of mean (S.E.M) and was subjected to one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using Graphpad prism version 5.0. Non-parametric Kruskal-Wallis test with Dunn's multiple comparison test was used for ulcer scores. Values of  $p < 0.05$  were considered significant.

## **CHAPTER FOUR**

### **RESULTS**

#### **4.1**

#### **Plant Yield**

About 2 kg of the powdered stem-bark of *K. senegalensis* gave 276.67 g (13.8%) of the crude methanol extract on complete extraction. Upon partitioning with hexane and ethylacetate, the crude methanol yielded 0.47 g (0.17%), 7.30 g (2.6%) and 62.21 g (22.5%) of *n*-hexane, ethylacetate and aqueous methanol fractions respectively.

#### **4.2**

#### **Phytochemical Screening of the Extracts**

Phytochemical analysis revealed the presence of carbohydrate, cardiac glycosides, saponins, flavonoids, steroids/triterpenes, alkaloids and tannins in the CME and AMF. The EAF contains all the metabolites except alkaloids while the HEF contains only steroids/triterpenes and flavonoids as shown in table 4.1.

Table 4.1 Phytochemical screening of the stem-bark extracts of *K. senegalensis*

<b>Metabolites</b>	<b>CME</b>	<b>HEF</b>	<b>EAF</b>	<b>AMF</b>
Carbohydrate	+	-	+	+
Cardiac glycosides	+	-	+	+
Anthraquinone	-	-	-	-
Saponins	+	-	+	+
Steroid and triterpenes	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	-	+	+
Alkaloids	+	-	-	+

**Key:**

Present = +

Absent = -

CME = Crude methanol extract

HEF = Hexane fraction

EAF = Ethyl acetate fraction

AMF = Aqueous methanol

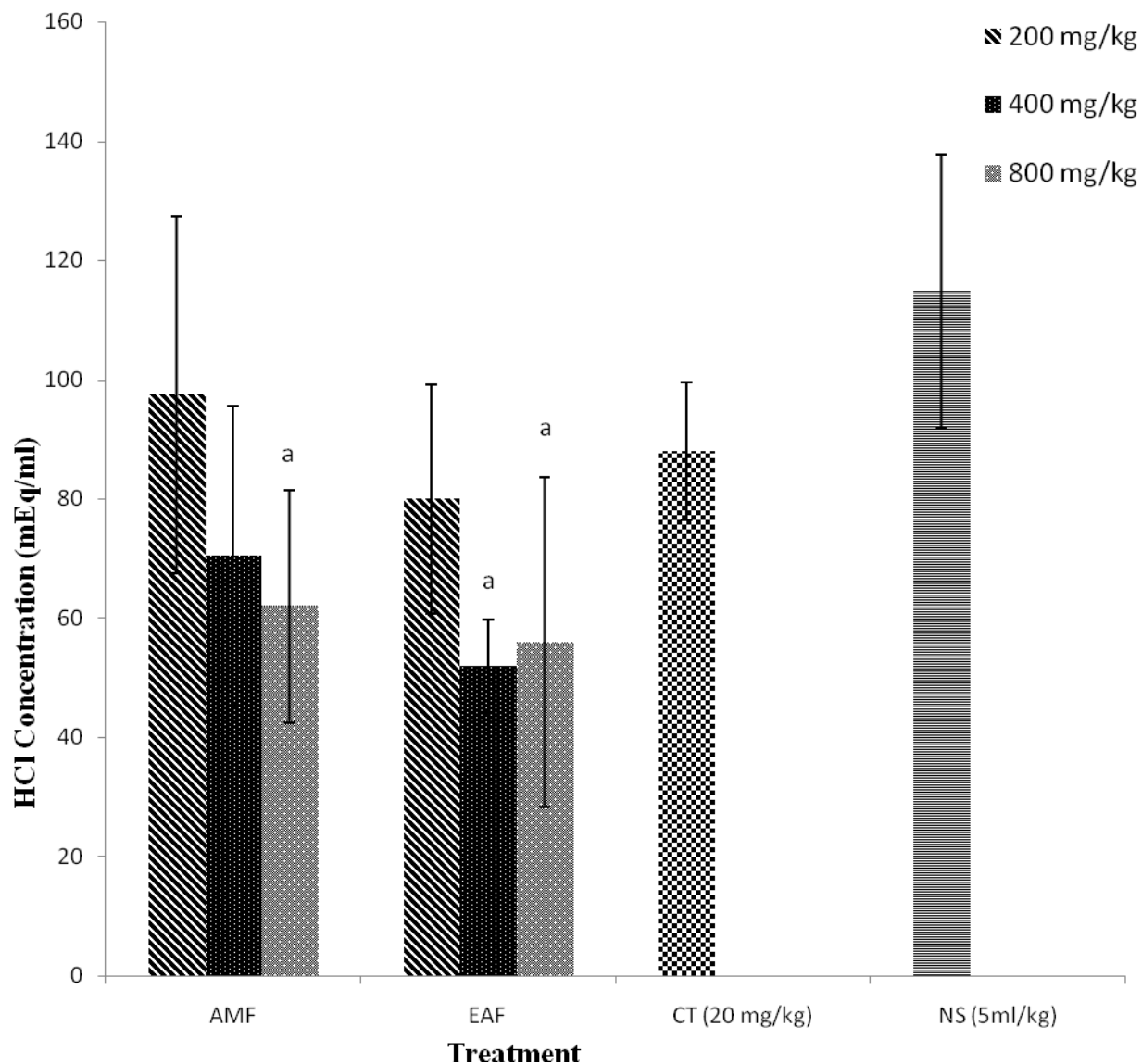
fraction

**4.3****Acute Toxicity Studies**

The *n*-hexane (HEF), ethylacetate (EAF) and the aqueous methanol (AMF) fractions did not produce any apparent toxic effect or mortality when tested at doses between 10 and 5000 mg/kg and the LD<sub>50</sub> was assumed to be > 5000 mg/kg.

**4.4****Effect of the Fractions of *Khaya senegalensis* on Gastric Acid**

There was a significant decrease ( $p < 0.05$ ) in the concentration of gastric HCl in rats treated with AMF (800 mg/kg) and EAF (400 and 800 mg/kg) relative to rats in the untreated control group. Although not significant, rats treated with AMF (200 and 400 mg/kg), EAF (200 mg/kg) and cimetidine (20 mg/kg) showed decreased levels of gastric HCl production as well (figure 4.1).



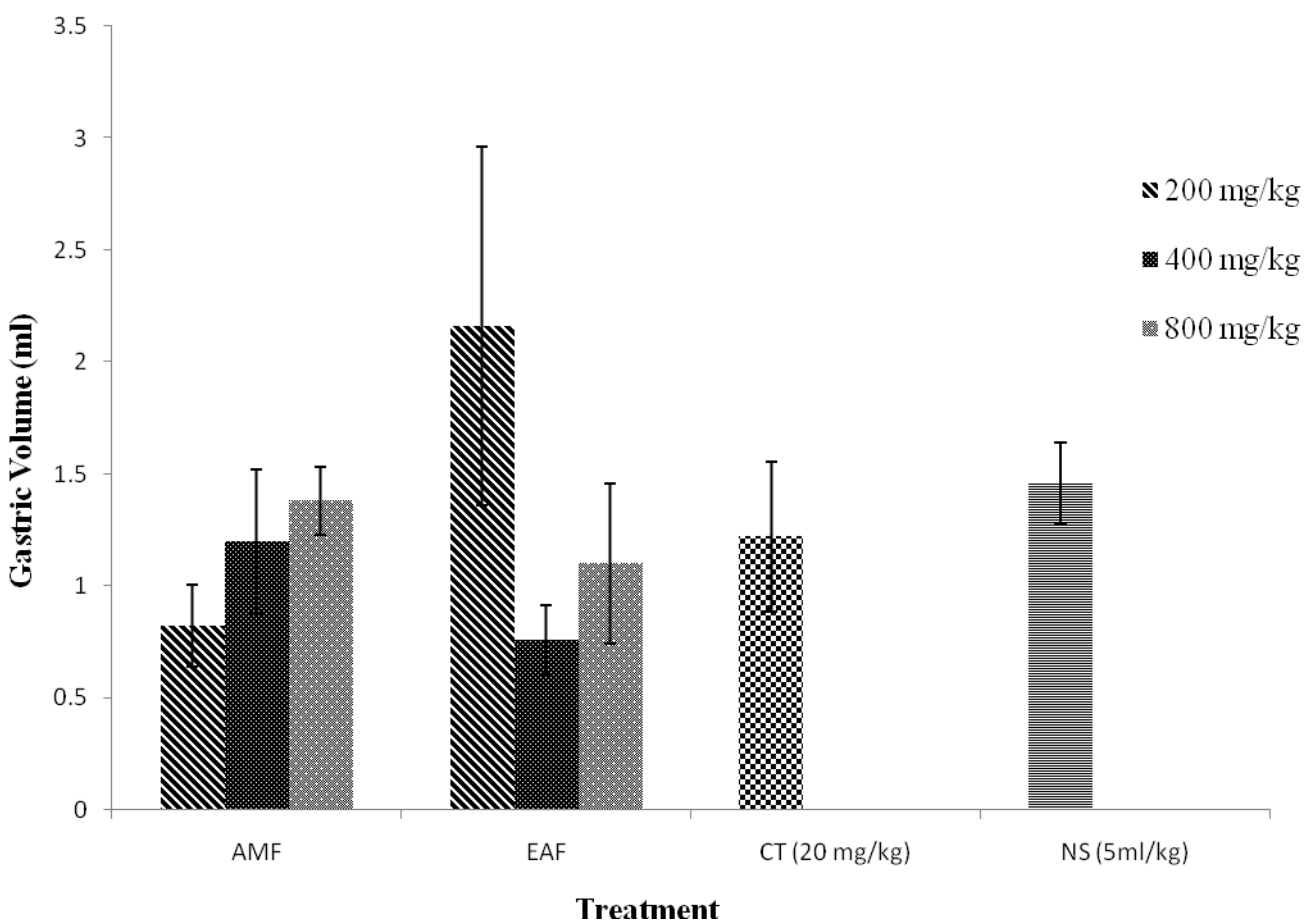
**Figure 4.1. The effect of the aqueous methanol (AMF) and ethyl acetate (EAF) fractions of *Khaya senegalensis* on gastric acid secretion in rats. Cimetidine (CT) and normal saline (NS) are used as treated and untreated controls respectively.**

Values are presented as mean  $\pm$  SEM. <sup>a</sup> $p < 0.05$  show significant difference when compared with normal saline untreated control group.

#### 4.5

#### Effect of the Fractions on Gastric Fluid

There was no significant difference in the volume of gastric fluid in rats treated with the AMF and EAF in all the tested doses when compared with the normal saline untreated group. Similarly, no significant difference was observed in the volume of gastric fluid in rats treated with cimetidine (20mg/kg) relative to the normal saline untreated group.



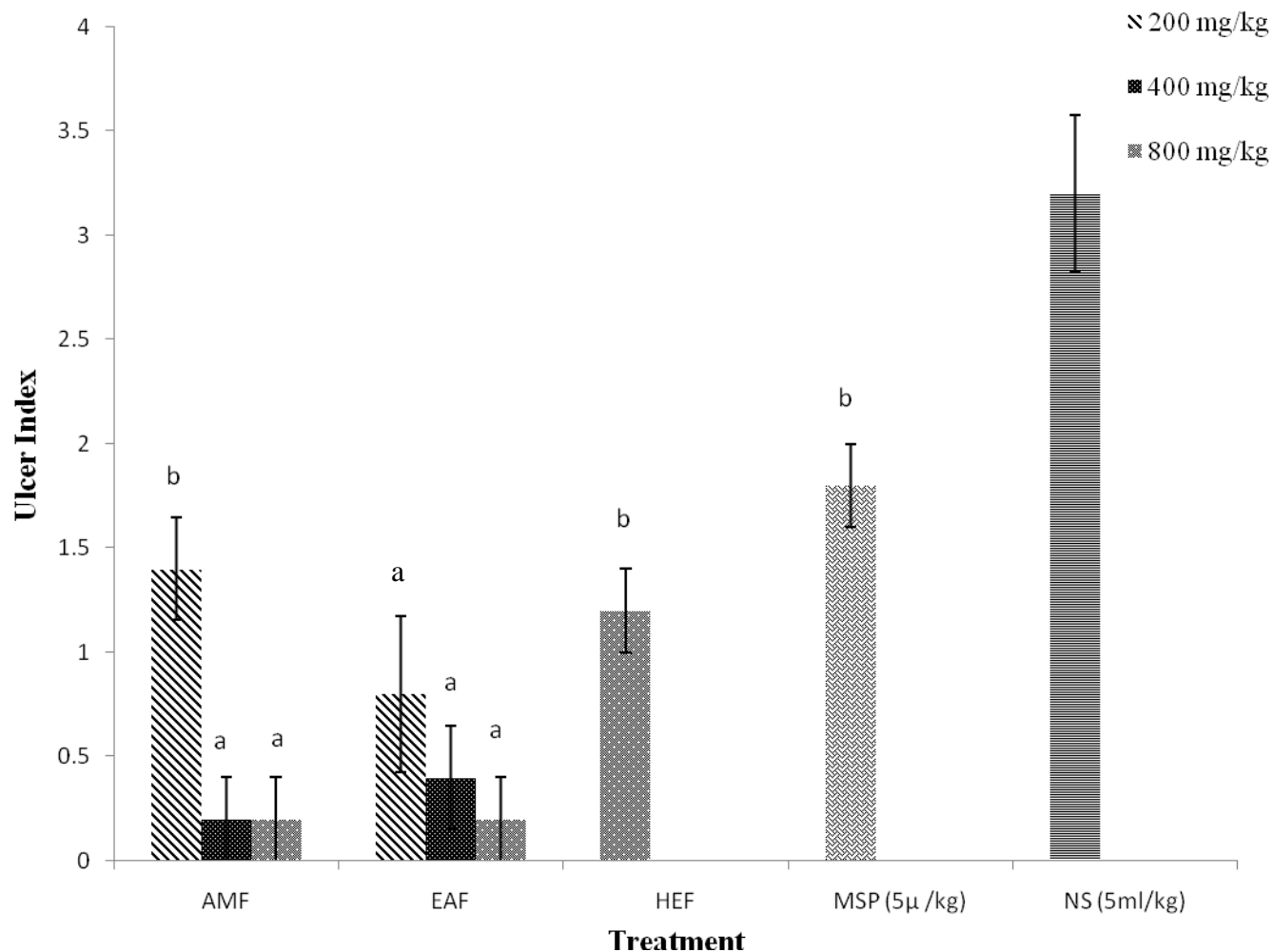
**Figure 4.2.** The effect of the aqueous methanol (AMF) and ethyl acetate (EAF) fractions of *Khaya senegalensis* on gastric fluid in rats. Cimetidine (CT) and normal saline (NS) are used as treated and untreated controls respectively.

Values are presented as mean  $\pm$  SEM.

#### 4.6 Effect of Fractions on Ethanol-induced Gastric Ulcer

The result of the ulcer indices of rats treated with different doses of the fractions of *K. senegalensis* are shown in Figure 4.3. The ulcer indices of rats treated with the AMF, EAF and

HEF were found to be statistically significant ( $p < 0.05$ ) when compared with the normal saline untreated group. Rats treated with the AMF and EAF at 400 and 800 mg/kg had significantly ( $p < 0.01$ ) lower ulcer index than those treated with the HEF and misoprostol (Standard drug).



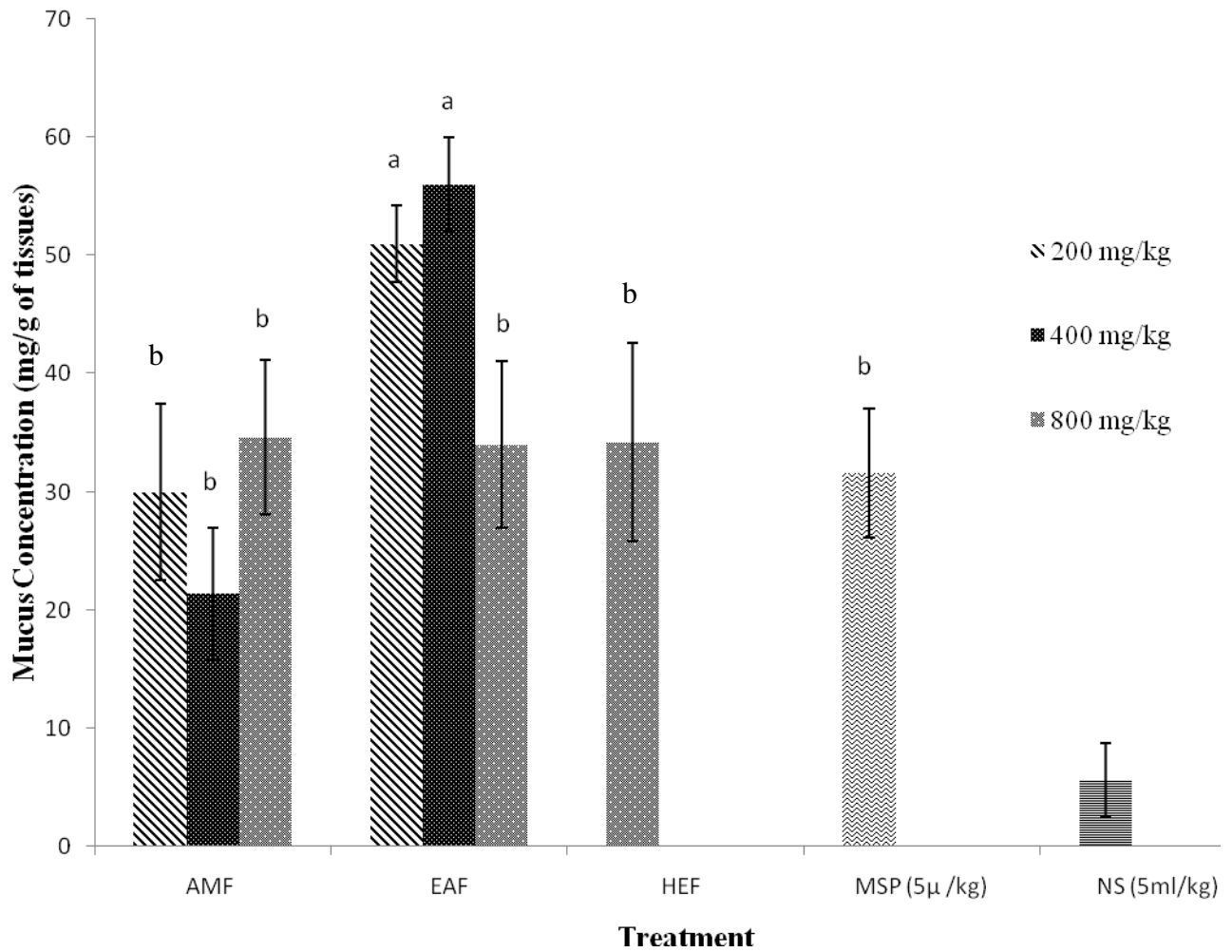
**Figure 4.3.** The ulcer protective effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* against ethanol-induced gastric ulceration in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls respectively.

Values are presented as mean  $\pm$  SEM. <sup>a</sup> $p < 0.01$ , <sup>b</sup> $p < 0.05$  show significant difference when compared with normal saline untreated control group.

#### 4.7

#### Effect of Fractions on Gastric Mucus Production

The effect of the fractions of *Khaya senegalensis* on gastric mucus production in rats is shown in Figure 4.4. There was a significant ( $p < 0.05$ ) increase in the concentration of gastric mucus in rats treated with the fractions (AMF, EAF and HEF) of *K. senegalensis* when compared with the normal saline untreated group. In a similar manner, rats treated with EAF (200 and 400 mg/kg) had a more significant ( $p < 0.01$ ) increase in mucus production when compared to all treated rats.

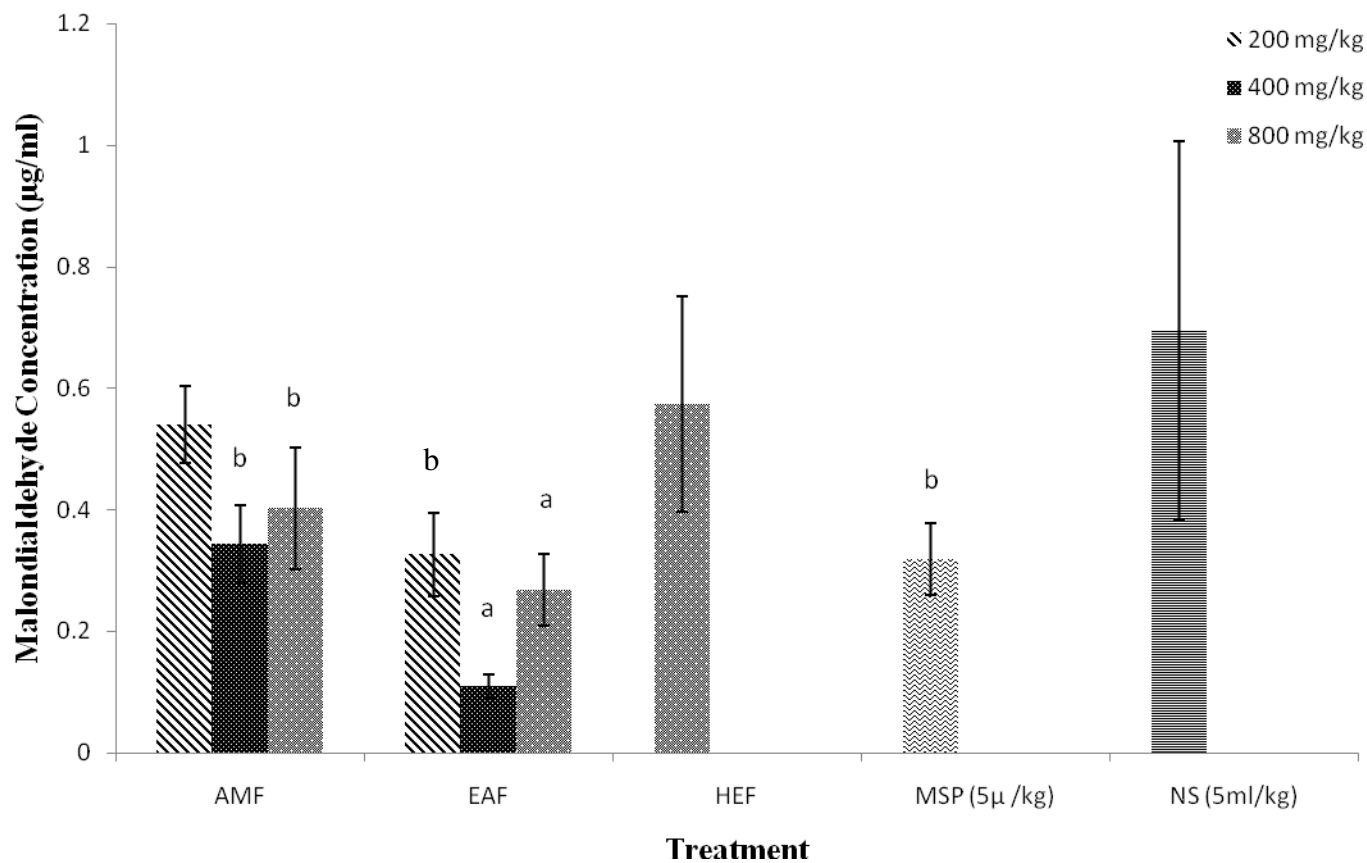


**Figure 4.4.** The effect of theaqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* on gastric mucus production in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls respectively.

Values are presented as mean  $\pm$  SEM. <sup>a</sup> $p < 0.01$ , <sup>b</sup> $p < 0.05$  show significant difference when compared with normal saline untreated control group.

#### **4.8 Effect of Fractions on Malondialdehyde Concentration**

The AMF, EAF and HEF significantly ( $p < 0.05$ ) reduced the concentration of malondialdehyde in the gastric tissues of treated rats (Figure 4.5). The AMF (400 and 800 mg/kg) and EAF (200 mg/kg) reduced significantly ( $p < 0.05$ ) the level of malondialdehyde in the stomach tissue of treated rats. Similarly, EAF (400 and 800 mg/kg) also reduced significantly ( $p < 0.01$ ) the level of malondialdehyde in the stomach tissue of rats when compared with the normal saline untreated group. Although, there was a decrease in the level of malondialdehyde in rats treated with HEF (800 mg/kg) it was not statistically significant.



**Figure 4.5.** The effect of the aqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* on gastric malondialdehyde concentration in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls respectively.

Values are presented as mean  $\pm$  SEM. <sup>a</sup> $p < 0.01$ , <sup>b</sup> $p < 0.05$  show significant difference when compared with normal saline untreated control group.

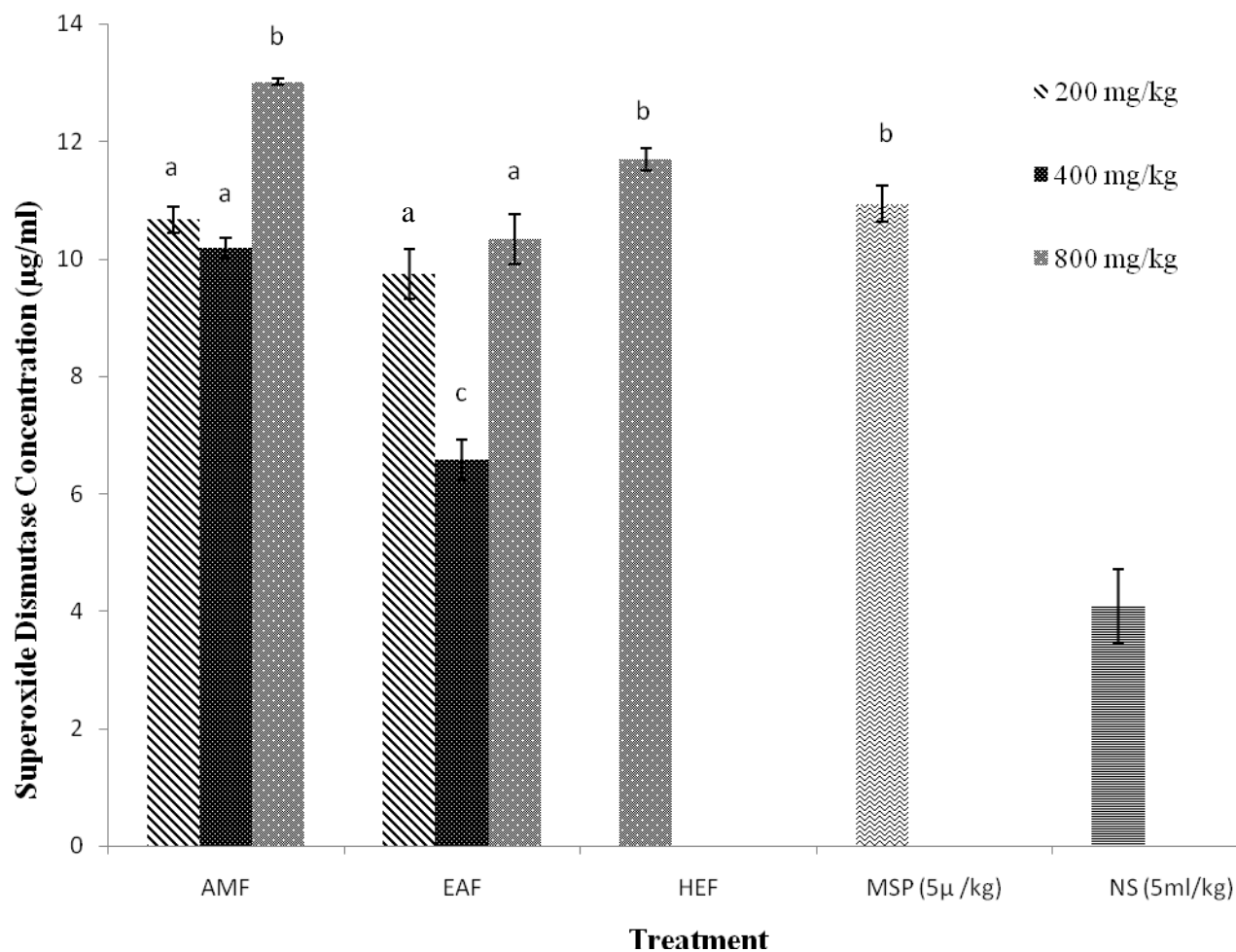
## 4.9

### Effect of Fraction on Stomach Antioxidant Enzymes

The superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes were assayed and their activities are shown (Figure 4.6 and Figure 4.7).

#### 4.9.1. Effects of the fractions on stomach superoxide dismutase activity

The fractions of *Khaya senegalensis* and misoprostol significantly ( $p < 0.05$ ) increased the activity of SOD (Figure 4.6). The activities of SOD in rats treated with AMF (200 and 400 mg/kg) and EAF (200 and 800 mg/kg) were significantly ( $p < 0.05$ ) higher when compared with the normal saline untreated group. Also HEF (800 mg/kg), AMF (800 mg/kg) and MSP (5  $\mu$ g/kg) produced a more significant ( $p < 0.01$ ) higher SOD activity in the treated rats. In addition, rats treated with EAF (400 mg/kg) had significant ( $p < 0.001$ ) increased in the level of SOD in their stomach tissue.

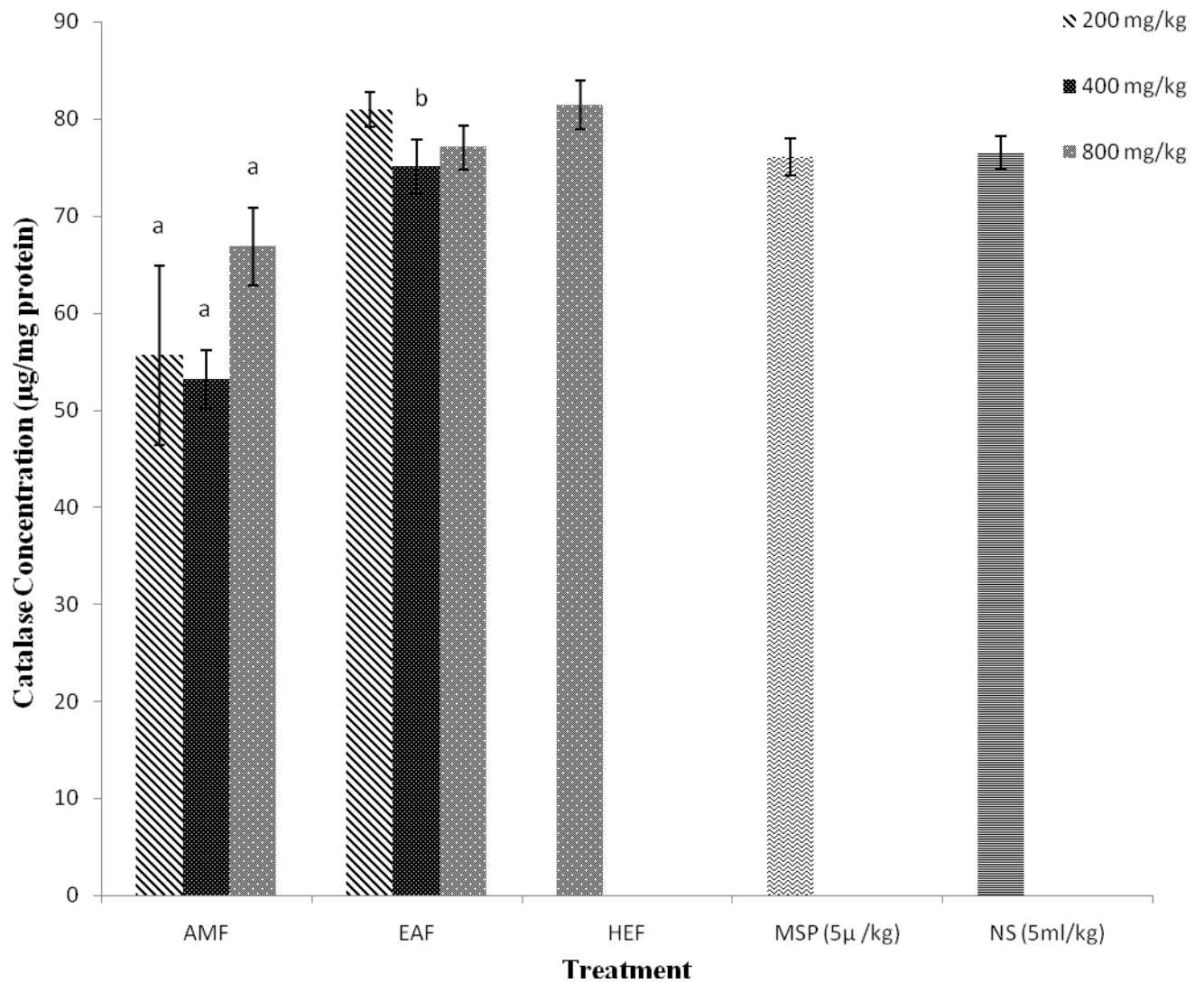


**Figure 4.6.** The effect of theaqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF)fractions of *Khaya senegalensis* on stomach superoxide dismutase activity in rats.Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls respectively.

Values are presented as mean  $\pm$  SEM. <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001 show significant difference when compared with normal saline untreated control group.

#### **4.9.2. Effect of fractions on stomach catalase activity**

The effect of the fractions of *Khaya senegalensis* on the activity of CAT is shown in Figure 4.7. The CAT activity for the AMF group was significantly ( $p < 0.01$ ) decreased when compared with the normal saline untreated group. The EAF treated group at the dose of 400 mg/kg also significantly ( $p < 0.05$ ) decreased the CAT activity when compared with the normal saline untreated group. There was no significant difference in the activity of CAT in the group treated with MSP (5  $\mu$ g/kg) when compared with the normal saline untreated group.

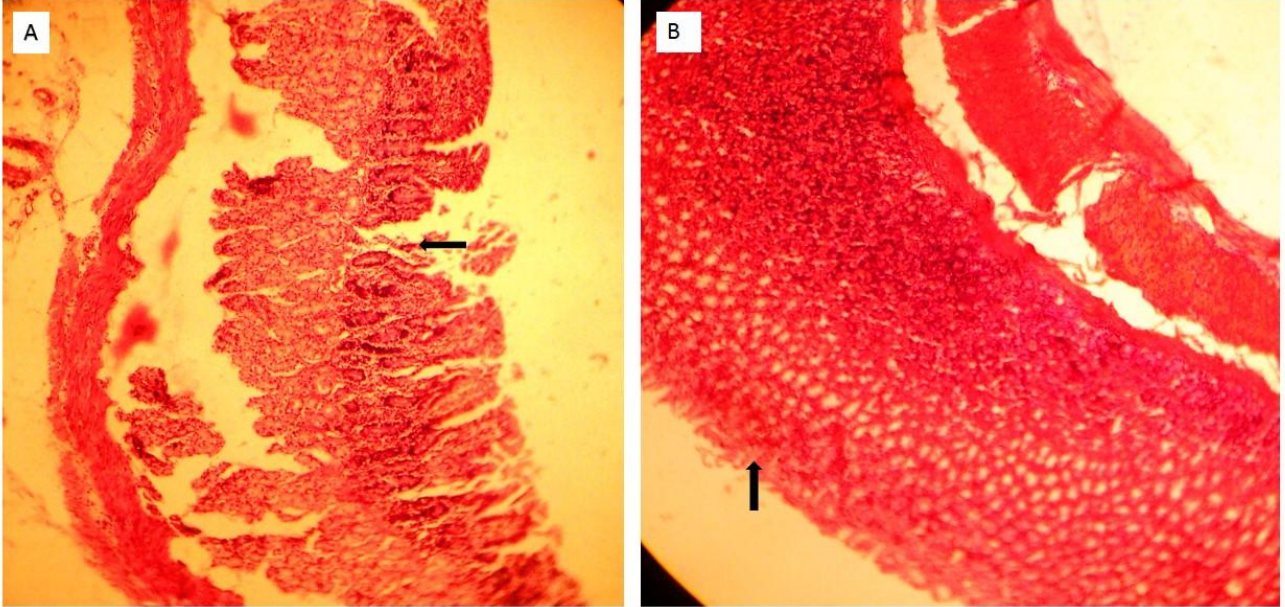


**Figure 4.7.** The effect of theaqueous methanol (AMF), ethyl acetate (EAF) and hexane (HEF) fractions of *Khaya senegalensis* on stomach catalase activity in rats. Misoprostol (MSP) and normal saline (NS) are used as treated and untreated controls respectively.

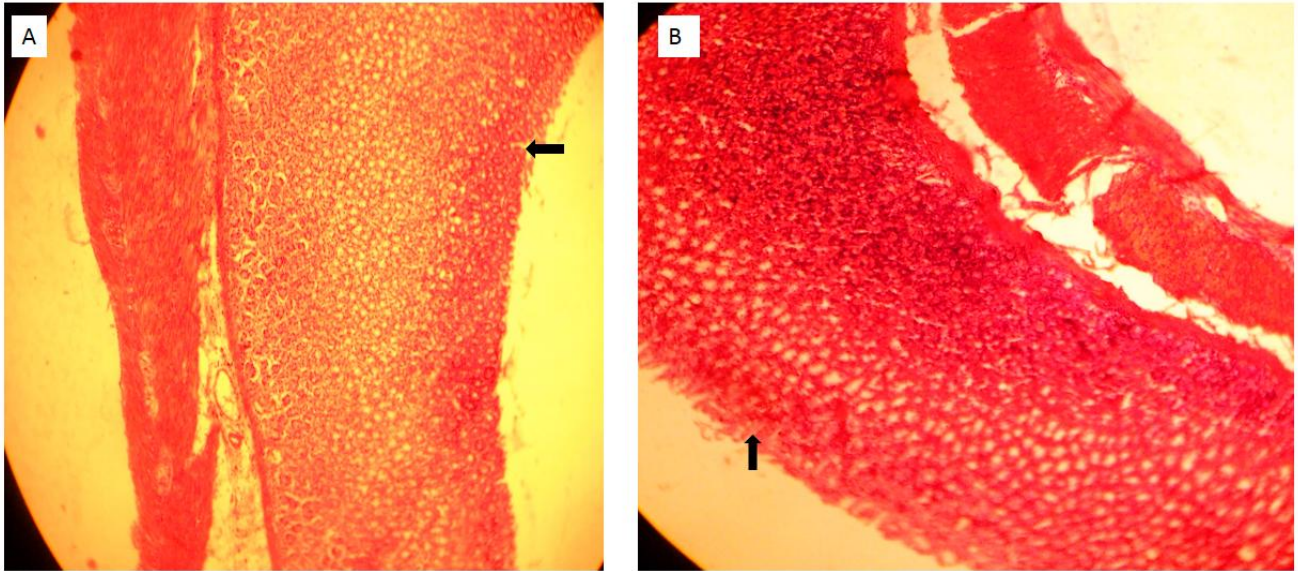
Values are presented as mean  $\pm$  SEM. <sup>a</sup>p<0.01, <sup>b</sup>p<0.05 show significant difference when compared with normal saline untreated control group.

#### **4.10 Effect of Fractions on Stomach Histo-architecture**

Histopathological changes were observed in the stomach of rats treated with AMF at the dose of 200 and 400 mg/kg (plates 2 and 4 respectively), but no changes were observed in the stomach of rats treated with AMF at the dose of 800 mg/kg (plate 6). The EAF treated groups at the dose of 200, 400 and 800 mg/kg showed milder lesions to the surface epithelium (plates 8, 10 and 12 respectively). Mild disruption to the surface epithelium were also observed in rats treated with HEF 800 mg/kg (plate 14). The normal saline untreated group showed severe necrosis and loss of epithelium lining (plate 18).

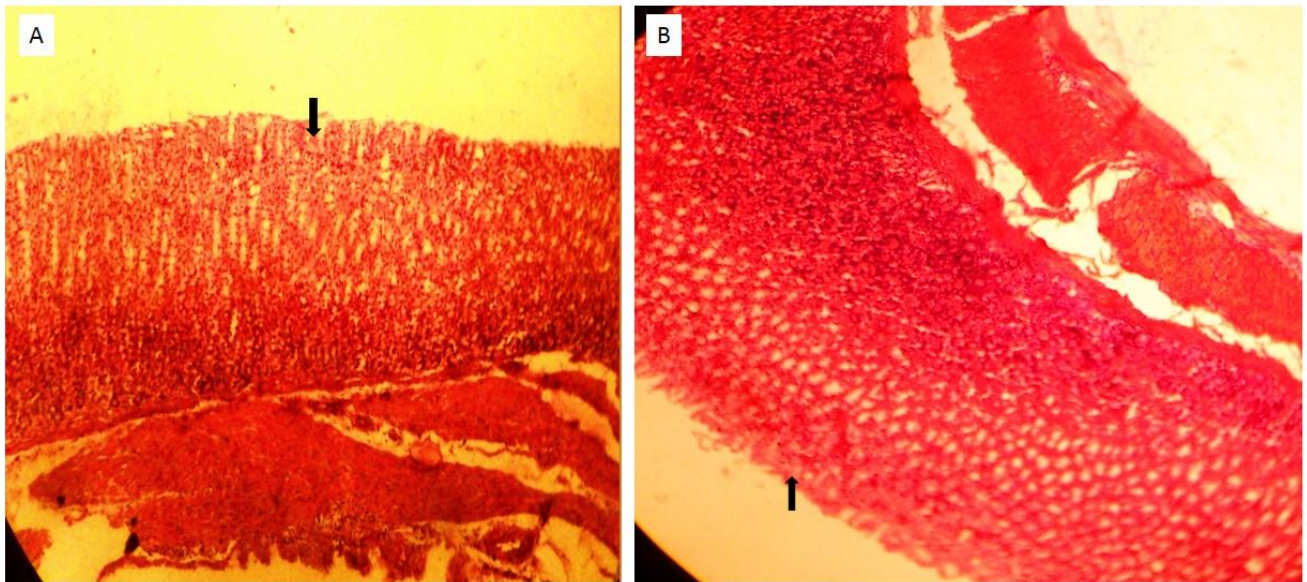


**Plate 2: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with aqueous methanol fraction of *Khaya senegalensis* (200 mg/kg) (A) showing necrosis and loss of epithelium lining (H and E  $\times$  100). (B) showing stomach of a normal rat.**

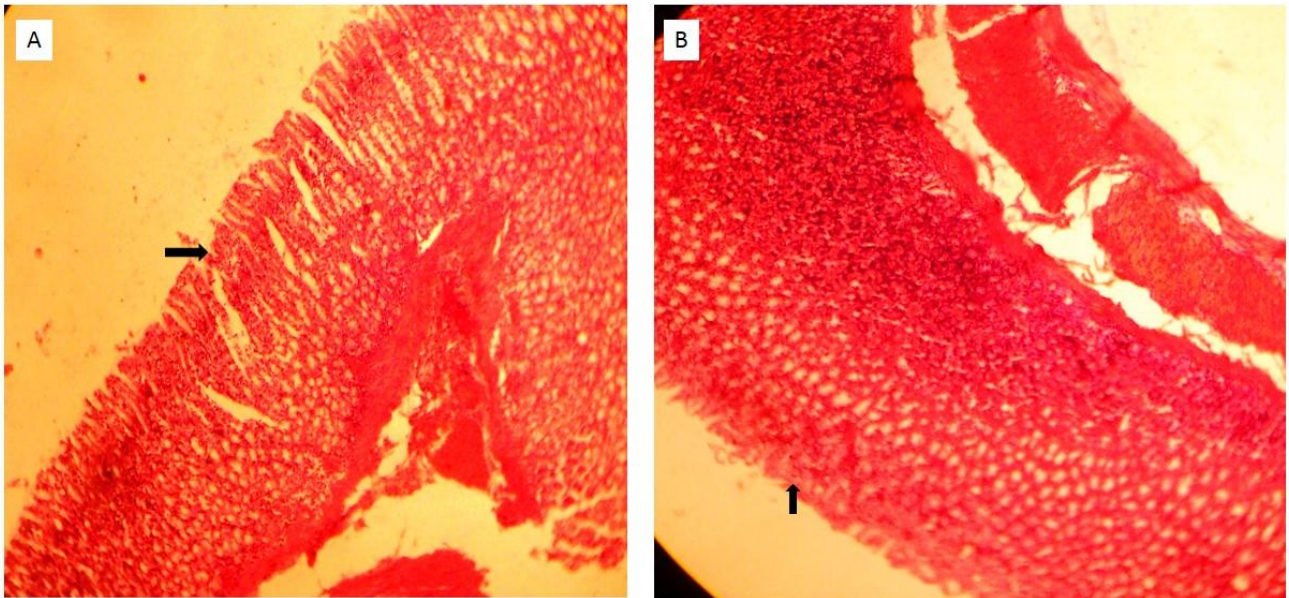


**Plate 3: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with aqueous methanol fraction of *Khaya senegalensis* (400 mg/kg) (A) showing mild disruption on the surface epithelium (H and E  $\times$  100). (B) Showing stomach of a normal rat.**



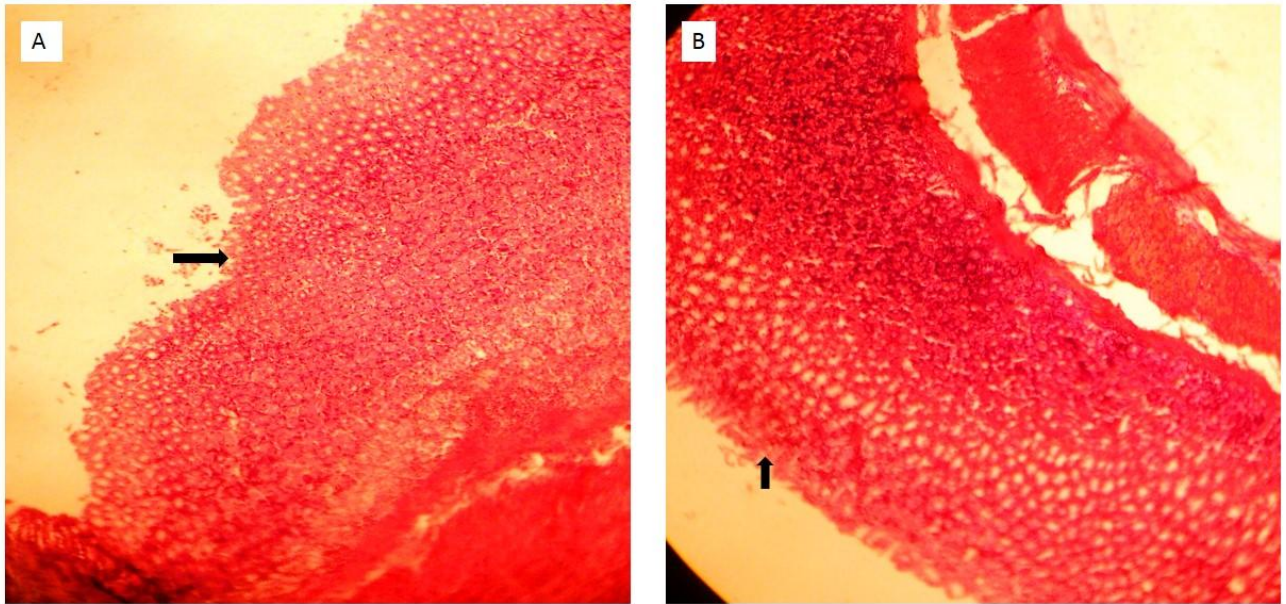


**Plate 4: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with aqueous methanol fraction of *Khaya senegalensis* (800 mg/kg) showing no disruption to the surface epithelium (H and E  $\times$  100). (B) Showing stomach of a normal rat.**

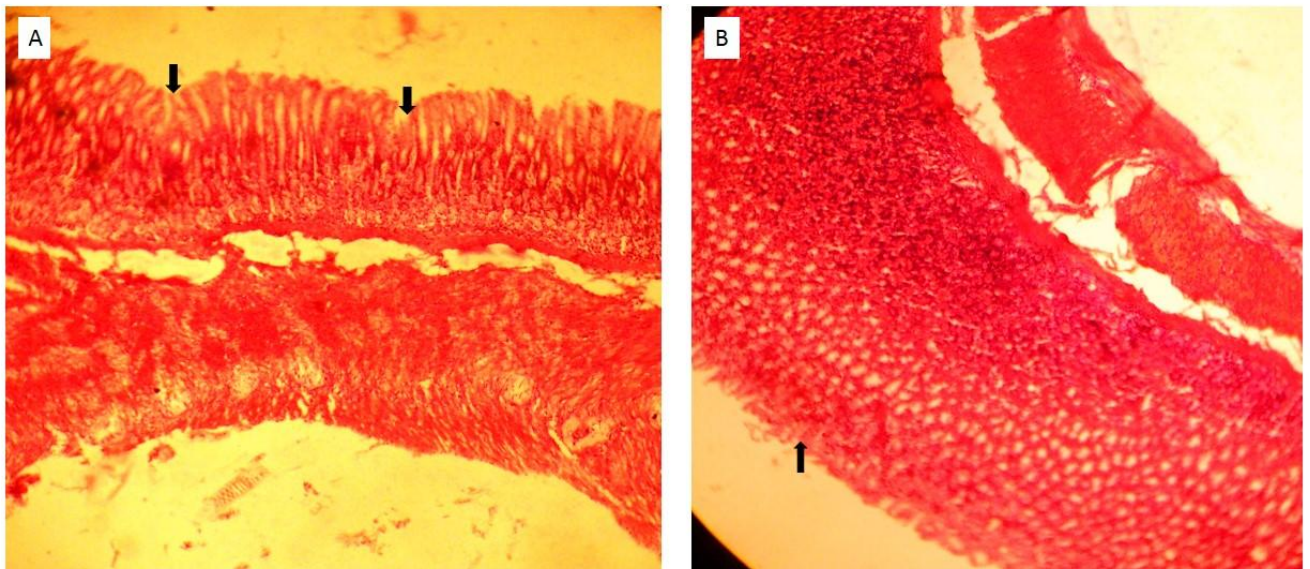


**Plate 5: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with ethylacetate fraction of *Khaya senegalensis* (200 mg/kg) showing mild excoriation of the epithelium lining (H and E  $\times$  100). (B) Showing stomach of a normal rat.**

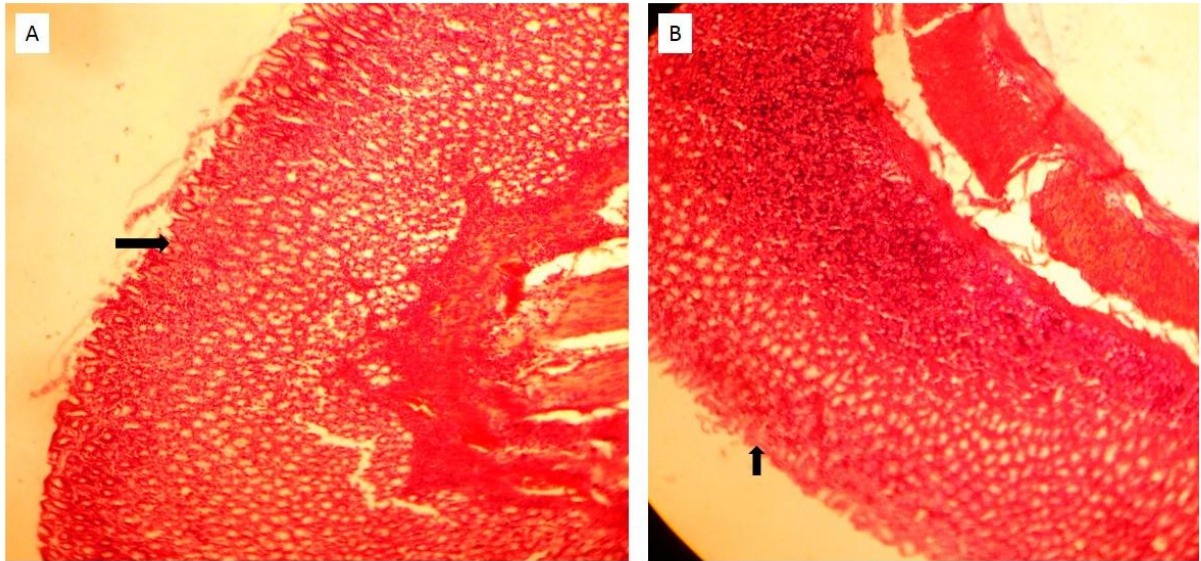




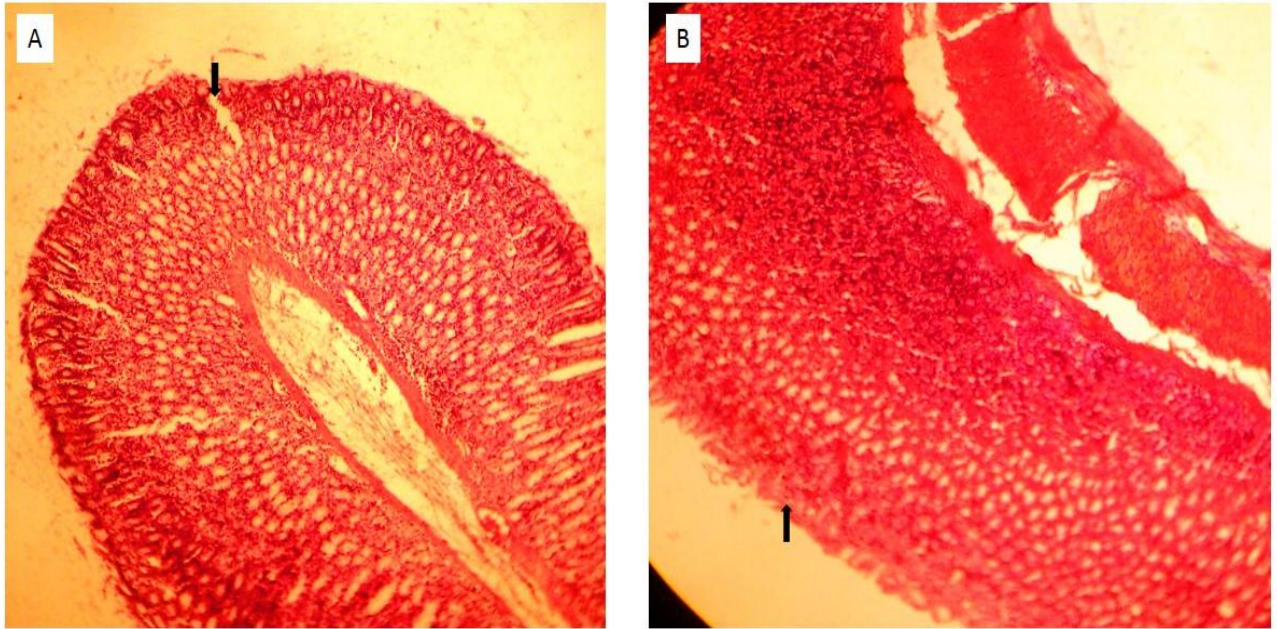
**Plate 6: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with ethylacetate fraction of *Khaya senegalensis* (400 mg/kg) showing loss of epithelium lining (H and E  $\times$  100). (B) Showing stomach of a normal rat.**



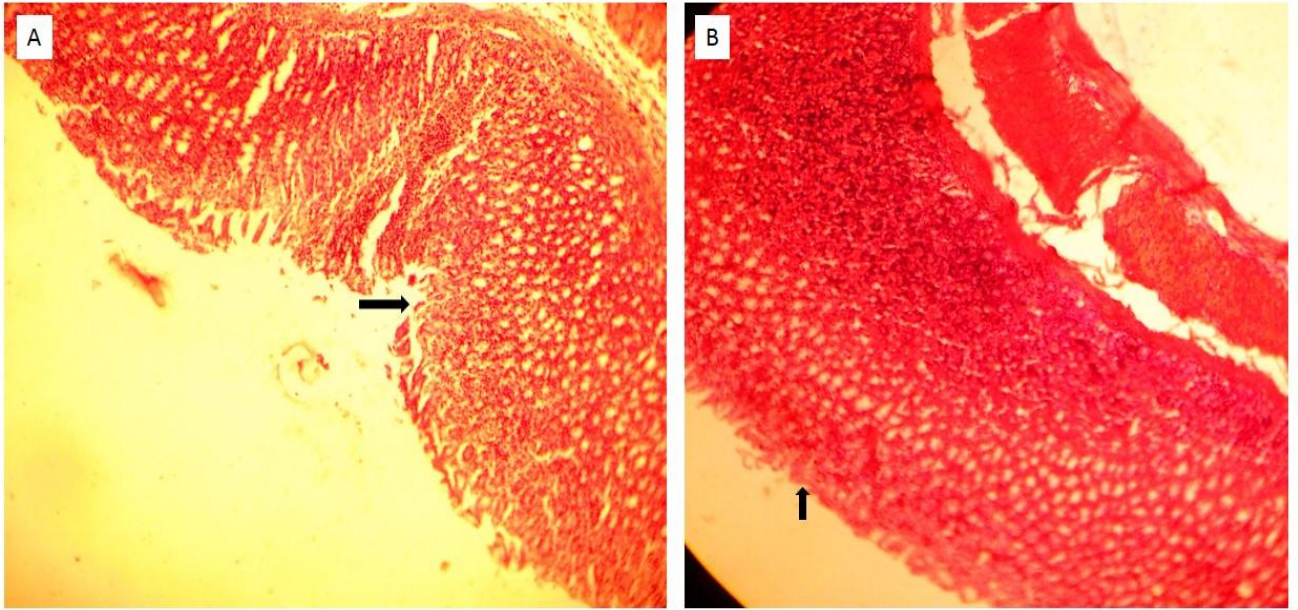
**Plate 7: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with ethylacetate fraction of *Khaya senegalensis* (800 mg/kg) showing mild loss of epithelium lining (H and E  $\times$  100). (B) Showing stomach of a normal rat.**



**Plate 8: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with hexane fraction of *Khaya senegalensis* (800 mg/kg) showing mild disruption to the surface epithelium (H and E  $\times$  100). (B) Showing stomach of a normal rat.**



**Plate 9: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with Misoprostol (5 µg/kg) showing mild excoriation to the surface epithelium (H and E × 100). (B) Showing stomach of a normal rat.**



**Plate 10: Photomicrograph of a section of the stomach of rat induced with gastric ulceration using 80% ethanol and treated with normal saline (5 ml/kg) showing severe necrosis and loss of epithelium lining (H and E  $\times$  100). (B) Showing stomach of a normal rat.**

## **CHAPTER FIVE**

### **5.0**

### **DISCUSSION**

Gastric ulcer is simply a defect in the mucosa of the stomach that penetrates the muscularis mucosa. But in the real sense, gastric and gastroduodenal ulceration described a clinical finding in which the cause is likely to be multifactorial and differ from one case to another. They are anatomical circumscribed breaks in the surface of gastrointestinal mucosa. The ulcer of the stomach or the proximal duodenum is also called peptic ulcers, because these ulcers are bathed by acid and pepsin (Parrah *et al.*, 2013). Gastroduodenal ulcers can occur independently or as a complication of many systemic diseases or following administration of various drugs to treat many diseases, therefore, it is important to understand its aetiological factors, pathophysiology for their effective treatment and early prophylaxis (Schaer, 2003).

*Khaya senegalensis* is a plant used in traditional medicine to treat and protect against gastric ulceration (Dalziel, 1956). The present study aimed to evaluate the anti-ulcer properties of the stem-bark fractions of *Khaya senegalensis*. Previous studies have shown that the methanol extract of the stem-bark of *Khaya senegalensis* possess anti-ulcer activity against ethanol induced gastric ulceration in rats (Suleiman *et al.*, 2013). In this study, the extracts of *Khaya senegalensis* afforded a significant protection against ulcers induced by pylorus ligation in rats. Pylorus ligation is one of the most widely used models for studying the effect of drugs on gastric acid and mucus secretion (Zainul *et al.*, 2014). Ulcers developed by ligating the pyloric end of the stomach are caused by an increase in gastric hydrochloric acid (HCl) secretion and/or stasis of acid, leading to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier (Kumar *et al.*, 2011). Parietal or oxyntic cells are the principal cell in gastric glands, which secrete gastric acid (HCl) to promote proteolytic digestion of foodstuffs, iron absorption, and killing pathogens (Rang *et al.*, 2012). The three most important mediators, namely acetylcholine, gastrin, and histamine, interact with specific receptors located at the basolateral

membrane of the parietal cells that stimulate gastric acid secretion (Oiry *et al.*, 1999). The regulation of gastric acid secretion by the parietal cells is an important factor in the pathogenesis of peptic ulcer. Therefore, the inhibition of gastric acid secretion is a key therapeutic target for the ulcer diseases (Jain *et al.*, 2007). Findings in this study demonstrated that the extracts of *Khaya senegalensis* significantly reduced the total acidity of the stomach in the rats. This may possibly be due to the anti-secretory property of the plant.

The HEF was tested at only 800 mg/kg in contrast to AMF and EAF that were evaluated at three different doses (200, 400 and 800 mg/kg). This was done because during the partitioning process we obtained very little quantity of HEF and therefore the maximum dose (800 mg/kg) used for the other extracts was chosen for evaluating the anti-ulcer activity of the fraction.

In gastric ulcers, in spite of low acid secretion, weakening of mucosal defenses can also lead to severe injury to the gastric mucosa (Jain *et al.*, 2007). The important criteria that determine the status of mucosal defense barrier against the unpleasant attack of acid and pepsin are the quality and quantity of gastric mucus secretion (Rachchh and Jain, 2008). According to Venables (1986), rise in amount of mucus secreted by the gastric mucosal cells prevent ulcer formation by acting as an effective barrier to the back diffusion of hydrogen ions, improving the buffering of gastric acid juice and reducing stomach wall friction during peristalsis. The mucus comprises of mucin-type glycoproteins that can be detected by amounts of alcian blue binding (Corne *et al.*, 1974). Our present study revealed that administration of the extracts of *Khaya senegalensis* was able to increase the amount of mucus secretion, supporting the point that one of the potential mechanisms of the gastroprotective effect elicited by *Khaya senegalensis* is as a result of enhancement of the gastric mucosal defense action. Treatment with the fractions obtained from the crude extract of *Khaya senegalensis* appears to significantly weaken the gastric aggressive

factors by decreasing the amount of gastric secretion and total acidity while enhancing the cytoprotective factor by strengthening the gastric mucosal barrier. This perhaps explains the anti-ulcer effect of the extracts of *Khaya senegalensis* in the pylorus ligation induced ulcer model, whereby the plant produced a significant protection as compared to the control group. Other than the imbalance between the aggressive and defensive factors, increase in free radicals and oxidative processes also strongly contributes to the ulcer disease. For this reason, it is hypothesized that the ability of an extract/compound to scavenge free radicals and elicit antioxidant properties could also be one of the pathways in which the extracts exert its protective effect. Pathogenesis of gastric ulcer involves oxidative stress with antioxidants playing a very important role in order to protect gastric mucosa (Trivedi and Rawal, 2001) and repairing gastric damage.

The inflammatory processes are thought to be responsible for producing various mediators, which are involved in the production of reactive oxygen species (ROS) and nitric oxide (NO) that contribute to the pathogenesis of ulcer disease (Ahlam *et al.*, 2016). Nitric oxide is a ubiquitous mediator and plays an important role as an endogenous modulator of numerous physiological functions. In the gastrointestinal tract (GIT), NO participates in the modulation of the smooth musculature tone, such as the regulation of intestinal peristalsis, gastric emptying, and antral motor activity (Martín *et al.*, 2001). It also helps in maintaining the gastric mucosal blood flow, barrier function, alkaline production, and regulates gastric mucus and acid secretion (Calatayud *et al.*, 2001). In physiological conditions, NO modulates both the integrity and repairing of the tissues (Martín *et al.*, 2001). This shows the double-edged role played by NO in gastrointestinal ulcerative and inflammatory diseases, Barrachina *et al.* (2001) have reviewed in detail the dual role of nitric oxide in modulating

gastrointestinal mucosal defense and injury. Therefore, the extracts of *Khaya senegalensis* may have exhibited its gastroprotective activity via modulation of NO.

Besides that, xanthine oxidase (XO) is an enzyme that generates ROS from the chemical reaction it catalyzes. ROS react with cellular lipids, causing the formation of lipid peroxides, which are metabolized to malondialdehyde, a major product of lipid peroxidation (Abdelwahab *et al.*, 2013). Huh *et al.* (1996) also reported that alcohol-induced gastric mucosal damage may be, in part, due to the increased activity of xanthine oxidase and the type of conversion rate of the enzyme, leading to oxidative stress. An endogenous antioxidant defense mechanism may constantly remove the continuous production of ROS during normal metabolism (Lemos *et al.*, 2012). Thus, the decrease seen in malondialdehyde concentration in the groups treated with the extracts of *Khaya senegalensis* could be due to moderate inhibition of xanthine oxidase exerted by the extracts which may have contributed to its antiulcerative effect via its antioxidant, especially the radical scavenging property as reported earlier in this study.

Phytochemical screening of the stem-bark extracts of *Khaya senegalensis* revealed the presence of flavonoids, saponins, steroids, tannins, alkaloids, cardiac glycosides and carbohydrates. Flavonoids have been reported to act in the gastrointestinal tract, exhibiting antispasmodic (Lima *et al.*, 2005), anti-secretory, antidiarrhoeal (Di Carlo *et al.*, 1993) and antiulcer properties (La Casa *et al.*, 2000). Flavonoids were also reported to exhibit antioxidant (Tapas *et al.*, 2008; Ferreira *et al.*, 2010) and anti-inflammatory (Sandhar *et al.*, 2011) activities. Flavonoids are able to activate the mucosal defense system through stimulation of gastric mucus secretion and scavenge for the ROS and free radicals produced by ethanol (Abdelwahab *et al.*, 2011). In addition, flavonoids are able to decrease ulcerogenic lesions by promoting the formation of gastric mucosa, inhibit the production of pepsinogen and diminish acid mucosal secretion (La

Casa et al., 2000). Kelly *et al.*, (2009) also reported that flavonoids have antihistaminic properties, thus, decreases histamine levels, as well as preventing the release of histamine from gastric mast cells, and inhibiting the gastric H<sup>+</sup>/K<sup>+</sup> proton pump and diminishing gastric acid secretion. Mota *et al.* (2009) have summarized the literature on 95 flavonoids with varying degrees of antiulcerogenic activity, confirming that flavonoids have a therapeutic potential for the more effective treatment of peptic ulcers.

Flavonoids are divided into five major subgroups, namely flavones, flavonols, flavanonols, flavanones and dihydroflavonols. Flavonoids like rutin, dihydroquercetin, fisetin and quercetin had been reported to prevent gastric mucosal ulceration in several animal models (La Casa *et al.*, 2000; Kahraman et al., 2003; Rao *et al.*, 2003). Izzo *et al.* (1994) reported that the protective effect of rutin was mediated by endogenous platelet-activating factor (PAF) and according to La Casa *et al.* (2000) the compound's antioxidant properties may be another possible mechanism underlying the antiulcer effect of rutin. Another study also revealed that rutin exerts antiulcer effect by inhibiting the gastric proton pump (Dubey *et al.*, 2013). On the other hand, quercetin's mechanism of action involves an increase in mucus production (Alarcón de la Lastra *et al.*, 1994), antihistaminic properties, inhibition of *Helicobacter pylori* (Beil *et al.*, 1995), and its antioxidant properties (Kahraman *et al.*, 2003). Quercetin was also reported to promote gastric healing (Motilva *et al.*, 1992). Barnaulov *et al.* (1982) demonstrated the antiulcer effect of quercetin in reserpin-induced ulceration whereas Kandhare *et al.* (2011) reported the therapeutic value of fisetin in the prevention of experimental gastric ulcer by virtue of its antioxidant mechanism.

Alkaloids such as atropine and scopolamine constitute an important class of anticholinergic compounds and clinically, they are used to block the muscarinic activity of acetylcholine thereby

showing antisecretory effects in the treatment of peptic ulcer (Maria *et al.*, 2008). On the other hand, tannins are known to protect the outer most layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation (Asuzu and Onu, 1990). Tannins form a protective pellicle by promoting precipitation of protein on the ulcer in order to prevent ulcer development. This pellicle helps in preventing toxic substance absorption and combat the attack of proteolytic enzymes (John and Onabanjo, 1990; Nwafor *et al.*, 1996). Furthermore, the saponins may exert its protective activities in ulceration by the activation of mucous membrane protective factors (Choudhary *et al.*, 2013).

SOD represents one aspect of protection against free radical mediated organ toxicity. Decrease SOD activity may cause many deleterious effect due to accumulation of superoxide radicals (Srivastava *et al.*, 2011). Flavonoids have been shown to increase the activity of SOD and other antioxidant enzyme. In addition they cause a decrease in activity of MDA (Jiang *et al.*, 2011). The increase observed in the SOD activity of the groups treated with the extracts of *Khaya Senegalensis* may be due to the antioxidant properties of the stem-bark extracts of the plant.

The decrease in CAT activity in rats treated with the extracts of *Khaya senegalensis* may be attributed to the possible binding of the enzymes to flavonoid components of the extracts. Flavonoids such as quercetin and myricetin bind to CAT and they alter the conformation of the enzyme (Zhu *et al.*, 2007). Krych and Gebicka (2013) also reported that flavonoids inhibit CAT activity, due to the formation of hydrogen bonds between catalase and flavonoids to form an unreactive catalase compound.

Considering all these reports on different substances found in the stem-bark extracts of *Khaya senegalensis*, it can be suggested that the gastroprotective activity of the stem-bark extracts of

*Khaya senegalensis* involved partly synergistic action of flavonoids, tannins, alkaloids and saponins.

## CHAPTER SIX

### 6.0 CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

In conclusion, this study has shown that:

- the AMF, EAF and HEF of *Khaya senegalensis* are relatively safe when administered to rats orally up to a dose of 5000 mg/kg.
- the AMF (800 mg/kg) and EAF (400 and 800 mg/kg) of *Khaya senegalensis* inhibit gastric acid secretions in treated rats.
- the AMF (200, 400 and 800 mg/kg), EAF (400 and 800 mg/kg) and HEF (800 mg/kg) of *Khaya senegalensis* increases the action of mucosal protective factors in rats experimentally induced with gastric ulceration.
- the AMF (400 and 800 mg/kg), EAF (200, 400 and 800 mg/kg) and HEF (800 mg/kg) of *Khaya senegalensis* have cytoprotective properties in rats experimentally induced with gastric ulceration.
- the AMF (400 and 800 mg/kg) and EAF (400 and 800 mg/kg) of *Khaya senegalensis* significantly decreases malondialdehyde activity in rats experimentally induced with gastric ulceration.
- the AMF (200, 400 and 800 mg/kg), EAF (400 and 800 mg/kg) and HEF (800 mg/kg) of *Khaya senegalensis* significantly increases superoxide dismutase activity in rats experimentally induced with gastric ulceration.

## 6.2

### Recommendations

- Further research should be carried out in order to isolate the compounds of the extracts of *Khaya senegalensis* responsible for the antiulcer properties and to elucidate their mechanisms of action.
- Detailed toxicity testing using both laboratory and target animal species should be carried on the extract of *Khaya senegalensis* to justify its clinical application.
- Phytochemical evaluation to determine whether these compounds act singly or jointly to exert the observed pharmacological effect will be worthwhile.

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### **Appendix 1: Total Acidity Aqueous Methanol Extract**

#### **Group 1 (200 mg/Kg)**

<b>Rat</b>	<b>Conc (Meq)</b>
1	62
2	178
3	107
4	43
5	7.3

#### **Group 2 (400 mg/Kg)**

<b>Rat</b>	<b>Conc (Meq)</b>
1	46
2	134
3	18
4	9.6
5	84

#### **Group 3 (800 mg/kg)**

<b>Rat</b>	<b>Conc (Meq)</b>	
	1	139
	2	32
	3	50
	4	49
	5	40

**Total acidity Ethyl acetate fraction  
Group 1 (200 mg/kg)**

<b>Rat</b>	<b>Conc (Meq)</b>	
	1	31
	2	70
	3	124
	4	324
	5	95

**Group 2 (400 mg/kg)**

<b>Rat</b>	<b>Conc (Meq)</b>	
	1	34
	2	38
	3	50
	4	62
	5	76

**Group 3 (800 mg/kg)**

<b>Rat</b>	<b>Conc (Meq)</b>	
	1	24
	2	34
	3	37
	4	19
	5	166

**Normal saline Group (5ml/kg)**

<b>Rat</b>	<b>Conc (Meq)</b>	
	1	139
	2	82
	3	70
	4	89
	5	194

**Cimetidine Group (20 mg/kg)**

<b>Rat</b>	<b>Conc (Meq)</b>	
	1	117
	2	68
	3	76
	4	115
	5	64

## **Appendix 2: Volume of Acid Aqueous Methanol Extract**

### **Group 1 (200 mg/kg)**

<b>Rat</b>	<b>Volume (ml)</b>	
	1	0.5
	2	0.9
	3	0.6
	4	0.6
	5	1.5

### **Group 2 (400 mg/kg)**

<b>Rat</b>	<b>Volume (ml)</b>	
	1	0.5
	2	0.8
	3	1.5
	4	2.3
	5	0.9

### **Group 3 (800 mg/kg)**

<b>Rat</b>	<b>Volume (ml)</b>	
	1	0.9
	2	1.5

3	1.5
4	1.8
5	1.2

**Volume of acid ethyl acetate fraction**

**Group 1 (200 mg/kg)**

<b>Rat</b>	<b>Volume (ml)</b>
1	4.9
2	2.6
3	2.2
4	0.5
5	0.6

**Group 2 (400 mg/kg)**

<b>Rat</b>	<b>Volume (ml)</b>
1	1.3
2	0.6
3	0.9
4	0.5
5	0.5

**Group 3 (800 mg/kg)**

<b>Rat</b>	<b>Volume (ml)</b>
1	0.9
2	0.8
3	2.5
4	0.8
5	0.5

**Normal saline Group (5ml/kg)**

<b>Rat</b>	<b>Volume (ml)</b>
1	1.3
2	1.8

	3	1.4
	4	1.9
	5	0.9
<b>Cimetidine Group (20mg/kg)</b>		
<b>Rat</b>	<b>Volume (ml)</b>	
	1	0.6
	2	1.1
	3	1.4
	4	0.6
	5	2.4

### Appendix 3: Ulcer Index

<b>Aqueous fraction</b>	<b>Score of lesions (ulcer index)</b>	
Group 1 (200mg/kg)		1.4
Group 2 (400mg/kg)		0.2
Group 3 (800mg/kg)		0.2
<b>Ethyl acetate fraction</b>	<b>ulcer index</b>	
Group 1 (200mg/kg)		0.8
Group 2 (400mg/kg)		0.4
Group 3 (800mg/kg)		0.2
<b>n-Hexane fraction</b>	<b>ulcer index</b>	
Group 1 (800mg/kg)		1.2
<b>Normal saline Group</b>	<b>ulcer index</b>	
(5ml/kg)		3.2
<b>Misoprostol Group</b>	<b>ulcer index</b>	
(5µg/kg)		1.8

#### Appendix 4: Gastric Malondialdehyde Determination

##### Aqueous fraction

##### Group 1 (200mg/kg)

Rat	MDA Conc ( $\mu\text{g/ml}$ )
1	0.59
2	0.41
3	0.56
4	0.4
5	0.74

##### Group 2 (400mg/kg)

Rat	MDA Conc ( $\mu\text{g/ml}$ )
1	0.23
2	0.31
3	0.57
4	0.39
5	0.22

##### Group 3 (800mg/kg)

Rat	MDA Conc ( $\mu\text{g/ml}$ )
1	0.26
2	0.3
3	0.33
4	0.8
5	0.32

##### Ethyl acetate fraction

##### Group 1 (200mg/kg)

Rat	MDA Conc ( $\mu\text{g/ml}$ )
-----	-------------------------------

	1	0.49
	2	0.1
	3	0.35
	4	0.43
	5	0.26

**Group 2 (400mg/kg)**

<b>Rat</b>	<b>MDA Conc (µg/ml)</b>	
	1	0.1
	2	0.16
	3	0.045
	4	0.1
	5	0.14

**Group 3 (800mg/kg)**

<b>Rat</b>	<b>MDA Conc (µg/ml)</b>	
	1	0.14
	2	0.14
	3	0.33
	4	0.28
	5	0.45

**n-Hexane fraction**

**Group 1 (800mg/kg)**

<b>Rat</b>	<b>MDA Conc (µg/ml)</b>	
	1	0.38
	2	0.58
	3	0.76
	4	1.1
	5	0.05

**Normal saline Group  
(5ml/kg)**

<b>Rat</b>	<b>MDA Conc (µg/ml)</b>	
	1	1.8
	2	0.01
	3	0.75
	4	0.71
	5	0.2

**Misoprostol Group  
(5µg/kg)**

<b>Rat</b>	<b>MDA Conc (µg/ml)</b>	
	1	0.43
	2	0.301

3	0.15
4	0.47
5	0.24

### Appendix 5: Gastric Mucus Determination

	Conc (µg/ml)
<b>Aqueous fraction</b>	
Group 1 (200mg/kg)	30
Group 2 (400mg/kg)	21
Group 3 (800mg/kg)	35
	Conc (µg/ml)
<b>Ethyl acetate fraction</b>	
Group 1 (200mg/kg)	52
Group 2 (400mg/kg)	61
Group 3 (800mg/kg)	34
	Conc (µg/ml)
<b>n-Hexane fraction</b>	
Group 1 (800mg/kg)	34
	Conc (µg/ml)
<b>Normal saline Group</b> (5ml/kg)	6
	Conc (µg/ml)
<b>Misoprostol Group</b> (5µg/kg)	32

## Appendix 6: Superoxide Dismutase (SOD)

### Aqueous fraction

#### Group 1 (200mg/kg)

Rat	SOD Conc ( $\mu\text{g/ml}$ )
1	10.34
2	11.16
3	10.61
4	11.16
5	10.07

#### Group 2 (400mg/kg)

Rat	SOD Conc ( $\mu\text{g/ml}$ )
1	10.07
2	10.61
3	10.34
4	10.34
5	9.52

#### Group 3 (800mg/kg)

Rat	SOD Conc ( $\mu\text{g/ml}$ )
1	13.06
2	13.06
3	13.06
4	12.79
5	13.06

### Ethyl acetate fraction

#### Group 1 (200mg/kg)

Rat	SOD Conc ( $\mu\text{g/ml}$ )
1	10.61
2	8.16
3	10.34
4	9.8

5 9.8

**Group 2 (400mg/kg)**

<b>Rat</b>	<b>SOD Conc (µg/ml)</b>
1	6.26
2	6.8
3	5.44
4	7.35
5	7.07

**Group 3 (800mg/kg)**

<b>Rat</b>	<b>SOD Conc (µg/ml)</b>
1	11.16
2	10.88
3	8.71
4	10.34
5	10.61

**n-Hexane fraction  
Group 1 (800mg/kg)**

<b>Rat</b>	<b>SOD Conc (µg/ml)</b>
1	11.97
2	12.24
3	11.43
4	11.16
5	11.7

**Normal saline Group  
(5ml/kg)**

<b>Rat</b>	<b>SOD Conc (µg/ml)</b>
1	2.72
2	3.81
3	3.81
4	3.54

5 6.53

**Misoprostol Group  
5µg/kg**

**Rat**

**SOD Conc  
(µg/ml)**

1	9.8
2	11.16
3	10.88
4	11.7
5	11.16

## Appendix 7: Catalase Activity (CAT)

### Aqueous fraction

#### Group 1 (200mg/kg)

Rat	CAT Conc ( $\mu\text{g}/\text{kg}$ )
1	73.17
2	29.27
3	56.1
4	41.46
5	78.05

#### Group 2 (400mg/kg)

Rat	CAT Conc ( $\mu\text{g}/\text{kg}$ )
1	48.78
2	56.1
3	60.98
4	43.9
5	56.1

#### Group 3 (800mg/kg)

Rat	CAT Conc ( $\mu\text{g}/\text{kg}$ )
1	51.22
2	68.29
3	70.73
4	73.17
5	70.73

### Ethyl acetate fraction

#### Group 1 (200mg/kg)

Rat	CAT Conc ( $\mu\text{g}/\text{kg}$ )
1	87.8
2	80.49
3	80.49
4	78.05
5	78.05

**Group 2 (400mg/kg)**

<b>Rat</b>	<b>CAT Conc (<math>\mu\text{g}/\text{kg}</math>)</b>
1	75.61
2	85.37
3	70.73
4	73.17
5	70.73

**Group 3 (800mg/kg)**

<b>Rat</b>	<b>CAT Conc (<math>\mu\text{g}/\text{kg}</math>)</b>
1	73.17
2	78.05
3	70.73
4	82.93
5	80.49

**n-Hexane fraction****Group 1 (800mg/kg)**

<b>Rat</b>	<b>CAT Conc (<math>\mu\text{g}/\text{kg}</math>)</b>
1	90.24
2	80.49
3	82.93
4	78.05
5	75.61

**Normal saline Group  
(5ml/kg)**

<b>Rat</b>	<b>CAT Conc (<math>\mu\text{g}/\text{kg}</math>)</b>
1	73.17
2	75.61
3	73.17
4	80.49
5	80.49

**Misoprostol Group  
(5 $\mu\text{g}/\text{kg}$ )**

<b>Rat</b>	<b>CAT Conc (<math>\mu\text{g}/\text{kg}</math>)</b>
1	75.61
2	70.73
3	80.49
4	80.49
5	73.17