GASTRO-INTESTINAL MUCOSAL PROTECTIVE PROPERTIES AND TOXICITY STUDIES ON THE METHANOL ROOT EXTRACT OF *WALTHERIA INDICA* LINN. (STERCULIACEAE) IN LABORATORY ANIMALS

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AUGUST, 2018

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**DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES AHMADU BELLO UNIVERSITY, ZARIA NIGERIA**

AUGUST, 2018

DECLARATION

I declare that the work in this dissertation entitled "**GASTRO-INTESTINAL MUCOSAL PROTECTIVE PROPERTIES AND TOXICITY STUDIES ON THE METHANOL ROOT EXTRACT OF *WALTHERIA INDICA* LINN. (STERCULIACEAE) IN LABORATORY ANIMALS**" has been performed by me in the Department of Pharmacology and Therapeutics under the joint supervision of Prof. S.S.Gyang, Prof. (Mrs.) H.O. Kwanashie and Dr. (Mrs.) L.O. Ayanwuyi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for the award of another degree or diploma at this or any other institution.

NGYAMA BLESSED BAGOBIRI-YEM	Signature	Date

CERTIFICATION

This dissertation entitled "**GASTRO-INTESTINAL MUCOSAL PROTECTIVE PROPERTIES AND TOXICITY STUDIES ON THE METHANOL ROOT EXTRACT OF *WALTHERIA INDICA* LINN. (STERCULIACEAE) IN LABORATORY ANIMALS**" by Ngyama Blessed BAGOBIRI-YEM meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to God almighty my strength, sustainer and ever present help and to my late father Dr. P.D. Bagobiri.

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ABSTRACT

Many countries worldwide including Nigeria use different parts of *Waltheria indica* (leaves, stem-barks, roots and whole plants) to treat various ailments. In Nigeria, *Waltheria indica* is used to treat gastro-intestinal ulcers, pain, inflammation, diarrhoea, conjunctivitis, anaemia and erectile dysfunction. The aim of this study was to evaluate the gastro-intestinal mucosal protective properties and ascertain the toxicity profile of the methanol root extract of *Waltheria indica.* Preliminary phytochemical screening was carried out on the extract and the oral median lethal dose (LD_{50}) of the extract was estimated using modified Lorke's (1983) method in rats followed by sub-acute toxicity study using OECD 407, 2008 guidelines where haematological and biochemical indices as well as histology examination of selected organs were carried out. The gastro-protective effect of the extract (250, 500 and 1,000 mg/kg) was evaluated using indomethacin-induced ulcer and pylorus ligation-induced ulcer models in Wistar rats and the extracts effect on isolated guinea pig ileum and rabbit jejunum was also determined. Phytoconstituents like alkaloids, cardiac glycosides, flavonoids, saponin glycoside, tannins and triterpenes were present in the methanol root extract of *Waltheria indica* and the oral LD_{50} was estimated to be greater than 5,000 mg/kg with no significant effect on the weekly body weights of the rats and relative organ weight ratio of the stomach, intestine, liver, kidney and heart. There was no significant effect on haematological parameters and renal function indices. However, there was statistically significant increase ($p \leq 0.05$) in alanine transaminase, total bilirubin and conjugated bilirubin at doses of (250 mg/kg), (250, 500 and 1,000 mg/kg) and (1,000 mg/kg) respectively. Histology examination of the liver, showed polymorphonuclear infiltration at doses of (250 and 1,000 mg/kg) and moderate hepatocellular necrosis at (500 mg/kg) with tubular necrosis observed in the kidney at 1,000 mg/kg extract dose. There was significant

($p \leq 0.05$) and dose dependent protection against indomethacin-induced ulcers in rats. The extract also significantly ($p \leq 0.05$) and dose dependently protected the rats against pylorus ligation-induced ulcer. The methanol root extract, neither contracted nor relaxed the guinea pig ileum and rabbit jejunum respectively. These findings, suggest that the methanol root extract of *Waltheria indica*, may be considered relatively safe and it possesses gastro-intestinal mucosal protective properties. This supports the folklore claims for the use of the plant in the management of peptic ulcer disease.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACh	Acetylcholine
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
AST	Aspartate transaminase
Cag	Cytotoxin-associated gene
cAMP	Adenosine 3,5,-cyclic monophosphate
CCK2R	Cholecytokinin receptor
Cl^-	Chloride ion
CO_2	Carbondioxide
Con.Bil	Conjugated Bilirubin
COX	Cyclo-oxygenase
DU	Duodenal Ulcer
ECL	Enterochromaffin- like
FOBC	Final organ bath concentration
GU	Gastric ulcer
H^+	Hydrogen ion
H2R	Histamine receptor
Hb	Haemoglobin
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate ion
H&E	Haematoxilin and Eosin

H^+/K^+-ATPase	Hydrogen-potassium adenosine triphosphatase
H. pylori	*Helicobacter pylori*
H2RA	Histamine receptor antagonist
K^+	Potassium ion
LD_{50}	Median lethal dose
MEWI	Methanol root extract of *Waltheria indica*
Na^+	Sodium ion
NSAIDs	Non-steroidal anti-inflammatory drugs
OH^-	Hydroxyl ion
PPI	Proton pump inhibitor
PU	Peptic Ulcer
PUD	Peptic Ulcer Disease
RBC	Red Blood Cell
ROW	Relative organ weight
SEM	Standard error of mean
T.Bil	Total bilirubin
TP	Total protein
Vac A	Vacoulating cytotoxin A
WBC	White blood cell
W. indica	*Waltheria indica*
w/w	Weight by weight

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

A peptic ulcer (PU) is a sore on the lining of the gastro-intestinal tract and the two most common types are gastric ulcers (GU) and duodenal ulcers (DU). Peptic ulcer disease (PUD) is caused by an imbalance between □aggressive factors□ such as hydrochloric acid (HCl), pepsin, refluxed bile, leukotrienes, reactive oxygen species, *Helicobacter pylori* (*H. pylori*), non-steroidal anti-inflammatory drugs (NSAIDs) and ‘defensive factors□ such as mucous-bicarbonate barrier, prostaglandins, mucosal blood flow, cell regeneration, antioxidants, nitric oxide and some growth factors like vascular endothelial growth factor (Hoogerwerf and Pasricha, 2006; Morsy and El-Sheikh, 2011; Sunil *et al.*, 2012). Although the pathogenesis of PUD is multifactorial, *H. pylori* is the most common cause of the disease. The organism is present in about 95% of duodenal ulcer patients and 70% of gastric ulcer patients (Fashner and Gitu, 2015). The prevalence of *H. pylori* infection varies greatly among countries and among population groups in the same country with a worldwide prevalence rate of about 40% in developed countries and over 80% in developing countries (Shi *et al.*, 2008; Khalifehgholi *et al*., 2013).

In 2015, the world wide prevalence of *H. pylori* positive people was approximately 4.4 billion with prevalence highest in Africa (79.1%), Latin America (63.4%) and Asia (54.7%) and lowest in North America (37.1%) (Hooi *et al*., 2017).

In northern Nigeria, *H. pylori* prevalence rate of about 95-100% was reported among patients with DU and about 60-90% in patients with GU (Mustapha *et al*., 2006; Tijjani and Umar, 2008). In South west Nigeria, Adeniyi *et al* (2012) reported that about 81.4% of

patients with dyspepsia, were infected with *H. pylori* using culture as diagnostic test and in south east Nigeria, about 58.3% of peptic ulcer patients were infected with *H. pylori* using serology test (Obiajuru and Adogu, 2013).

Other causes and risk factors of PUD include: NSAIDs, stress, alcohol intake, smoking, hereditary and environmental factors (Sowande and Aldrink, 2011). The complications of PUD are haemorrhage, penetration, perforation and gastric outlet obstruction.

Regardless of the etiology of PUD, the goals of therapy are to relieve symptoms, to heal and prevent reoccurrence and to prevent complications (Bhowmik *et al.*, 2010; Gadekar *et al.*, 2010).

Due to the side effects of the existing drugs used to treat PUD, some medicinal plants have been used over the years to treat the disease. The widely used plant is liquorice (*Glycyrrhiza glabra*). Others include garlic (*Allium sativum*), ginger (*Zingiber officinale*) and turmeric (curcuma longa) (Shmuely *et al*., 2016).

1.2 Statement of Research Problems

PUD and its complications contribute significantly to the health care burden worldwide (Boyapati *et al*., 2014). It is the most prevalent gastro-intestinal disorder and it is among the leading cause of morbidity in Nigeria and many developing countries and in Nigeria, its prevalence is more common among elderly people (Agbakwuru *et al.*, 2006; Adeniyi *et al*., 2012). The complications of PUD (haemorrhage, perforation, penetration and gastric outlet obstruction) lead to hospitalization if not detected and treated early (Boyapati *et al*., 2014; Fashner and Gitu, 2015). The side effects, inaccurate dosing and drug interactions of existing anti-ulcer drugs such as antacids, histamine-2 receptor antagonists (H2RA), proton pump inhibitors (PPIs), cytoprotectives and some antimicrobials has limited their efficacy (Sakat *et al*., 2012). Antacids cause constipation (Aluminium hydroxide) and diarrhoea

(Magnesium tricilicate) (Altman, 2001). Cimetidine, the prototype H2RA causes gynaecomastia, reduced sperm count and impotence in males, galactorrhoea in females and all H2RA interact with the cytochrome P450 enzyme system (Hoogerwerf and Pasricha, 2006). Inhibition of gastric acid release by PPIs leads to pernicious anaemia due to reduced absorption of vitamin B_{12} and loss of the physiological antimicrobial barrier provided by gastric acid which can lead to risk of enteric infection with *Clostridium difficile*, Salmonella and Campylobacter (De la Coba *et al*., 2016). Epstien *et al* (2006) reported that PPIs cause hypomagnesemia. This finding has been backed by recent studies by Perazella, 2013 and Misra *et al.,* 2015. Cytoprotectives like misoprostol, in addition to its dose-dependent diarrhoeal side effect, also has uterotropic effect therefore contraindicated in pregnancy. In addition to the above mentioned side effects of anti-PUD drugs, poor compliance, antibiotic resistance and wrong medication timing also contributes to their reduced effectiveness. Therefore, the need to carry out more research to discover plants that have gastro-protective properties cannot be over emphasized. Bioactive constituents from plants have been used for centuries to cure various ailments and the use of these phytochemical constituents as drug therapy to treat PU has proven to be clinically effective and relatively less toxic than existing drugs (Sen *et al*., 2009). This shows that there is a need to carry out research into more plants in order to discover these substances that can protect against ulcer and accelerate ulcer healing.

1.3 Justification for the Study

Many local communities in Nigeria use medicinal plants for treatment of different ailments. This poses a problem of incorrect dosage and subsequent adverse effects due to lack of proper regulation and knowledge of the toxicities of such medicinal plants (Alli *et al*.,

2015). There is a continuous need to carry out research into these useful medicinal plants used in the therapeutic management of peptic ulcers to ascertain their efficacy and safety. Also, the current treatment of peptic ulcer is focused on cure rather than prevention of the disease thus more research on medicinal plants known to have gastro-intestinal protective properties is encouraged as prevention is better than cure. PUD is an important cause of morbidity and mortality throughout the world affecting millions of people. The lifetime likelihood of developing PU is about 10% for males and 4% for females (Siddique, 2014). PUD is uncommon in children in the developed world, but has a prevalence rate of about 2% among children with abdominal pain in developing countries (Sowande and Aldrink, 2011). Natural products from plants are well known promising sources for the discovery of new anti-PUD agents as many of these plants are used in folk medicine for the treatment of PUD (Sakat *et al.,* 2012). Despite the many available drugs used to treat PUD, most of them are expensive and have different side effects. Thus, screening plants for bioactive constituents is still important and might provide a useful source of new gastro-protective compounds for developing pharmaceutical drugs or alternatively, as simple dietary adjuncts to existing therapies (Manoharan *et al*., 2010).

Waltheria indica (*W. indica*) has been used in the treatment of PUD in folk medicine (personal communication), but there was no scientifically evaluated evidence on its gastro-protective potential. Therefore, this work was designed to investigate the gastro-intestinal mucosal protective properties and toxicities of methanol root extract of *W. indica*.

1.4 Aim and Objectives

The aim of this research work is to investigate the gastro-intestinal mucosal protective properties and elaborate on the toxicities of methanol root extract of *W. indica* in some laboratory animals.

The objectives are as follows:

To carry out investigation on the preliminary phytochemical constituents of the methanol root extract of *W. indica.*

To conduct acute and sub-acute toxicity studies on the methanol root extract of *W. Indica*

To carry out indomethacin-induced ulcer and pylorus ligation-induced ulcer studies on the methanol root extract of *W. Indica*

To determine the effect of methanol root extract of *W. indica* on gastro-intestinal smooth muscle contractile activity of guinea pig ileum and rabbit jejunum.

1.5 Statement of Research Hypothesis

The methanol root extract of *W. indica* does not have gastro-intestinal mucosal protective activity and no effect on gastro-intestinal smooth muscle contractile activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Alimentary Canal

The alimentary canal or gastro-intestinal tract provides the body with a continual supply of water, electrolytes, and nutrients. Food is broken down mechanically in the mouth and movement is by peristalsis through the oesophagus to the stomach were digestive juices digest the food which is then absorbed in the intestine, circulated in blood and excreted through the anus (Guyton and Hall, 2000).

The stomach is a J shaped organ which secretes about 2.5 litres of gastric content daily. It has two curvatures: The greater curvature and the lesser curvature. Anatomically, the stomach is divided into four regions: the cardia, the fundus, the body and the gastric antrum. The pylorus is a tubular structure joining the stomach to the duodenum (Ganong, 2001). The stomach mucosa has two important types of tubular glands: oxyntic glands (also called gastric glands) and pyloric glands. The oxyntic glands secrete hydrochloric acid (HCl), pepsinogen, intrinsic factor, and mucous while the pyloric glands secrete mucous and the hormone gastrin (Kansara and Sakhreliya, 2013). A typical stomach oxyntic gland is composed of three types of cells: mucous neck cells, which secrete mainly mucous, peptic (or chief) cells, which secrete large quantities of pepsinogen and parietal (or oxyntic) cells, which secrete HCl and intrinsic factor. The antrum secretes gastrin from G cells (Thomson and Hunt, 2012). Choi *et al* (2014) documented that in addition to the G cells and mucous neck cells found on the gastric antrum, parietal cells and chief cells were also found in antral glands.

The small intestine absorbs digested food through the villi into the capillaries. It consists of the duodenum, the jejunum and the ileum. The duodenum is a hollow jointed tube connecting the stomach to the jejunum. It is the first and shortest part of the small intestine. It is also where bile and pancreatic juices enter the intestine. The jejunum is located between the distal end of duodenum and the proximal part of the ileum. It is covered with many tiny finger-like projections called villi which increase the surface area of tissue available to absorb nutrients. The ileum absorbs vitamin B_{12} and bile salts and is also crucial in the absorption of fat-soluble vitamins (Vitamin A, D, E and K) (Ganong, 2001).

The large intestine consists of the cecum, colon, rectum and anal canal. The principal functions of the colon are absorption of water and electrolytes from chyme to form solid feaces and storage of faecal matter until it can be expelled (Guyton and Hall, 2000).

2.2 Gastric Secretions

2.2.1 Hydrochloric acid (HCl)

HCl is formed inside intracellular canaliculi of the parietal cells. Water dissociates to form hydrogen ions (H^+) and hydroxyl ions (OH^-). The OH^- formed in this process reacts with carbondioxide (CO_2) (formed either during metabolism in the cell or entering the cell from the blood) to form bicarbonate ion (HCO_3^-). The HCO_3^- is transported out of the basolateral membrane in exchange for chloride ion (Cl^-). The outflow of HCO_3^- into the blood results in a slight elevation of blood pH known as the "alkaline tide". This process serves to maintain intracellular pH in the parietal cell. Cl^- and potassium (K^+) ions are transported into the lumen of the canaliculus. The H^+ formed from dissociation of water is pumped out of the cell, into the canaliculi, in exchange for K^+ catalysed by hydrogen-potassium adenosine triphosphatase (H^+/K^+ ATPase) also called the proton pump. This leaves the

canaliculus with a strong solution of HCl which is then secreted outwards through the open end of the canaliculus into the lumen of the gland (Guyton and Hall, 2000).

2.2.2 Intrinsic factor

Intrinsic factor is also secreted by the parietal cells of the stomach along with HCl. It is essential for the absorption of cyanocobalamin (vitamin B_{12}) in the ileum. When the parietal cells are destroyed, pernicious anaemia can develop. Vitamin B_{12} is essential for maturation of red blood cells in the bone marrow (Guyton and Hall, 2000).

2.2.3 Pepsinogen

Pepsinogen is the inactive precursor of pepsin the enzyme that digests proteins and it is secreted via the gastric chief cells (Kansara and Sakhreliya, 2013). When it is first secreted, it has no digestive activity but as soon as it comes in contact with HCl and previously formed pepsin, it is activated to form active pepsin. The rate of secretion of pepsinogen is strongly influenced by the amount of acid in the stomach (Guyton and Hall, 2000). Pepsin is activated by acid pH of about 1.8 to 3.5, reversibly inactivated at pH 4 and irreversibly destroyed at pH 7 (Kansara and Sakhreliya, 2013).

2.2.4 Gastrin

Gastrin is a hormone secreted by G cells located in the antrum of the stomach (Thomson and Hunt, 2012).When protein containing foods reach the antral end of the stomach, some of the protein from the food have a direct stimulatory effect on the G cells to cause release of gastrin (Guyton and Hall, 2000). In large doses, it has a variety of actions but its main action is the stimulation of gastric acid and pepsin secretion. It does this by stimulating the secretion of histamine from enterochromaffin like (ECL) cells. Gastrin secretion is inhibited by acid in the antrum partly by release of somatostatin a potent inhibitor of gastrin

secretion and partly by a direct action on the G cells. Increased secretion of gastrin increases acid secretion but a negative feedback occurs to inhibit further gastrin secretion (Ganong, 2001).

2.2.5 Prostaglandin

Prostaglandins are naturally occurring unsaturated fatty acids produced from arachidonic acid via cyclooxygenase enzyme. Prostaglandin E_2 is the principal prostaglandin synthesized in the stomach and it is an important gastro-protective mediator. Prostaglandins stimulate mucous and bicarbonate secretion, cause vasodilation of mucosal blood vessels thereby increasing mucosal blood flow, increase the resistance of epithelial cells to cytotoxins-induced injury and suppress the recruitment of leukocytes into gastric mucosa (Kansara and Sakhreliya, 2013).

2.2.6 Mucous

The entire surface of the stomach mucosa has a continuous layer of surface mucous cells. These cells secrete large quantities of viscid insoluble mucous which coats the stomach wall in a layer of about two millimetres thick (Guyton and Hall, 2000). Mucous is alkaline therefore the stomach wall is not directly exposed to the highly acidic stomach secretion. The slightest contact with food and/or especially any irritation of the mucosa directly stimulates the surface mucous cells to secrete additional copious quantities of thick, alkaline, viscid mucous (Guyton and Hall, 2000).

2.2.7 Bicarbonates

The surface mucous cells in addition to secreting mucous also secrete bicarbonate ions. The bicarbonate ion is trapped in the mucous gel (Ganong, 2001).

2.2.8 Somatostatin

Somatostatin is an endocrine hormone secreted by the D cells of the antrum and it inhibits the secretion of gastrin which leads to inhibition of gastric acid secretion (Tso, 2009).

2.3 Stimulation of Gastric Secretion

Gastric acid secretion is a complex, continuous process in which nervous and hormonal mechanisms contribute to a common endpoint: the secretion of HCl by parietal cells. Neuronal (acetylcholine), paracrine (histamine), and endocrine (gastrin) factors all regulate acid secretion. Their specific receptors muscarinic, histamine and cholecytokinin (M_3, H2, and CCK2 receptors respectively) are on the basolateral membrane of parietal cells in the body and fundus of the stomach (Hoogerwerf and Pasricha, 2006). They bind to their specific receptors and these receptors activate the secretory processes.

2.3.1 Acetylcholine (ACh)

The vagus nerve from the parasympathetic nervous system plays a central role in the regulation of gastric acid secretion and gastrin release. Efferent fibers originating in the dorsal motor nuclei descend to the stomach *via* the vagus nerve and synapse with ganglion cells of the enteric nervous system. ACh released from postganglionic vagal fibers directly stimulates gastric acid secretion through M_3 receptors on the basolateral membrane of parietal cells (Hoogerwerf and Pasricha, 2006). When ACh binds to its receptors, phospholipase C activated rise in calcium results in the elaboration of H^+ / K^+ ATPase in the parietal cell and this leads to the secretion of H^+ (Schubert and Peura, 2008). ACh contributes about 10% to total gastric output in guinea pigs under basal conditions (Bitziou and Patel, 2012).

2.3.2 Histamine

Histamine is released by ECL cells which are located within the gastric glands of the stomach. The histamine released, binds to H2-receptors (H2R) on the parietal cells and this causes activation of adenylate cyclase and generation of adenosine 3,5,-cyclic monophosphate (cAMP) (Schubert and Peura, 2008). Histamine release is halted when the pH of the stomach starts to decrease (Guyton and Hall, 2000). Under basal conditions, Bitziou and Patel (2012) observed that histamine contributes about 35% to total gastric output in guinea pigs.

2.3.3 Gastrin

Gastrin is a hormone produced in the G cells of the gastric antrum. It is the main stimulant of acid secretion during meal ingestion. It binds to CCK2 receptors (CCK2R) and this activates phospholipase C to induce release of cytosolic calcium. These receptors are also found on ECL cells so gastrin stimulates the parietal cells directly by activation of CCK2R and more importantly indirectly by releasing histamine from ECL cells (Schubert and Peura, 2008). The histamine released, then stimulates acid secretion through activation of the parietal H2R. Bitziou and Patel (2012) in their study on gastric acid and histamine release in guinea pigs observed that under basal conditions, direct or indirect gastrin induced gastric secretion accounted for about 55% of the total acid output.

2.4 Peptic Ulcer Disease

PUD is a group of disorders characterized by the presence of lesions in any portion of the GIT exposed to gastric acid in concentrations that can cause erosion (Pahwa *et al.*, 2010).

The pathophysiology of PUD is best viewed as an imbalance between mucosal defence factors (bicarbonate, mucous, prostaglandin, nitric oxide, some peptides and growth

factors) and injurious factors (HCl, pepsin, *H. pylori* and NSAIDs) (Hoogerwerf and Pasricha, 2006).

Other causes of PUD include NSAIDs, Zollinger-Ellison syndrome, gastro-oesophageal reflux disease (GERD) and oxidative stress. Physical stress, smoking and alcohol intake increase PU incidence (Bhat, 2013).

2.4.1 Types of peptic ulcers

2.4.1.1 Oesophageal ulcer

Oesophageal ulcer is defined as a break in the oesophageal mucosa. Its main cause is GERD and Pill oesophagitis caused by drugs like doxycycline, tetracycline and potassium chloride can be prevented by drinking plenty of water and sitting in an upright position while taking the drugs (Higuchi *et al.,* 2003; Dag *et al*., 2014). Human Immuno-Deficiency Virus (HIV) positive patients are prone to oesophageal ulcers due to suppression of their immune system by the virus which gives rise to opportunistic infections like infection with cytomegalovirus. Some signs and symptoms include: odynophagia, dysphagia and chest pain (Epstein and Locketz, 2009).

2.4.1.2 Gastric ulcer (GU)

Gastric ulcer is commonly found on the lesser curvature near the antrum and extends to an area of about 2–3 cm above the pylorus (Thomson and Hunt, 2012). Epigastric pain usually occurs during meal because as food enters the stomach, gastric acid production is increased (Mustafa *et al.,* 2015). The pain is usually accompanied by haematemesis and weight loss. It is common in both males and females but occurs more in the elderly (Pahwa *et al*., 2010; Bhat, 2013).

2.4.1.3 Duodenal ulcer (DU)

Duodenal ulcer is usually found in the duodenal bulb, the pyloric channel or pre-pyloric area (Thomson and Hunt, 2012). The pain is usually felt about 2-3 h after a meal when the stomach begins to release digested food and acid into the duodenum and is relieved by food in the stomach as acid does not reach the duodenum because the pyloric sphincter closes to concentrate the stomach contents (Mustafa *et al.,* 2015). DU is common in males and frequently accompanied with malena and weight gain (Bhat, 2013).

The above mentioned ulcers can either be acute or chronic. Chronic ulcers penetrate through the epithelium of the muscle layers of the gut wall and may affect some organs like the pancreas and liver (Pahwa *et al.,* 2010).

2.4.1.4 Idiopathic ulcer

Idiopathic ulcer is defined as an ulcer with unknown cause. Diagnosis involves thorough exclusion of other possible causes, detailed review of patient's medication history and careful evaluation of *H. pylori* infection by multiple tests (Chung *et al*., 2015). Idiopathic PUD is an independent risk factor associated with recurrent ulcer (Yoon *et al*., 2013).

2.4.2 Signs and symptoms of peptic ulcer disease

Epigastric pain: This pain may be felt anywhere from the navel up to the breast bone and can last for a few minutes to several hours. It may be worse when the stomach is empty especially at night and is often temporarily relieved by eating certain foods that buffer gastric acid or by taking antacids. The burning pain usually disappears and then returns for a few days or weeks (Bhowmik *et al.,* 2010).

Others include: nausea, vomiting, haematemesis, dark or tarry stool and loss of appetite.

2.4.3 Causes of peptic ulcer disease

2.4.3.1 Helicobacter pylori

H. pylori is a Gram negative, microaerophilic spiral bacteria and is the major cause of gastritis and PUD (Sowande and Aldrink, 2011). It was initially named *Campylobacter pyloridis* then *C. pylori* before it was placed in its own genus *Helicobacter* (Khalifa *et al*., 2010) and it is about 2.4 to 4.0 µm long and 0.5 to 1.0 µm wide (Brown, 2000). *H. pylori* is transmitted through the oral-oral or faecal-oral route (Goh *et al.*, 2011) and it is the major cause of PUD as well as other gastro-intestinal malignancies like gastric cancer, mucosal associated lymphoid tumours and extra-gastric disorders like iron deficiency anaemia (Vilaichone *et al*., 2013; Wong *et al*., 2014). Its outer membrane contains phospholipids, lipopolysaccharides, glycoproteins, cholesterol, glucosides and proteins (putative adhesion, porins, iron transporters, flagellum associated proteins and proteins of unknown function) (Akram *et al.*, 2010). It has about 4-6 flagella for movement, adherence to surface epithelia and penetration of the mucous layer. It also produces enzymes like urease, catalase, proteases, lipases and phospholipases. Urease cleaves urea to form ammonia and CO_2, making the environment surrounding it relatively alkaline. This facilitates its survival when it is exposed to low pH in the gastric lumen. Catalase, is an enzyme that protects against the damaging effects of oxygen metabolites, especially hydrogen peroxide released from leukocytes while proteases, lipases and phospholipases, hydrolyze the surface mucous layer and facilitate penetration into the mucous coat (Magaji *et al.*, 2008). *H. pylori* consist of a large diversity of strains with two phenotypes of the bacterium type 1 and type 2. Both make a vacuolating cytotoxin (Vac A). Type I also has a cytotoxin-associated gene (cag A) that codes for one of the major *H. pylori* virulence proteins and is associated with ulcer

formation. Type II organisms lack cag A and do not produce as much of an inflammatory response. Therefore, bacterial strains that have the cag A gene are associated with an ability to cause ulcers (Akram *et al.*, 2010; Pahwa *et al.*, 2010). *H .pylori* was first discovered in the stomachs of patients with gastritis and stomach ulcers in 1982 by physician Dr. Barry Marshall and pathologist Dr. Robin Warren of Perth, Western Australia. To demonstrate that *H. pylori* caused gastritis, Marshall drank a beaker of *H. pylori* culture and became ill several days later. An endoscopy ten days after inoculation revealed signs of gastritis and the presence of *H. pylori* which he treated with antibiotics and the symptoms resolved. This result suggested that *H. pylori* was the causative organism of gastritis and PUD (Richardson, 2006; Akram, *et al.*, 2010).

2.4.3.2 Non steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are drugs used in the management of pain and musculoskeletal disorders like arthritis. Unfortunately, their use has been limited by their association with the development of PUD and its complications (Lanza *et al.*, 2009). They cause gastro-duodenal mucosal injury through their inhibition of mucosal prostaglandin production. Prostaglandins promote mucosal integrity through several mechanisms such as: maintenance of mucosal blood flow, promoting mucosal bicarbonate and mucous formation and reducing mucosal acid secretion (Rostom *et al.*, 2011). NSAIDs inhibit cyclooxygenase (COX), which is the rate-limiting enzyme required for the conversion of arachidonic acid to prostaglandins (Pahwa *et al.*, 2010). COX-1 and COX-2 are the two known forms of COX. COX-1 plays an important role in gastro-intestinal mucosal protection, renal blood flow regulation and normal platelet function. In contrast, COX-2 is thought to generate prostaglandins that are responsible for pain and inflammation. Non-selective NSAIDs

inhibit both COX-1 and COX-2 pathways. NSAIDs with higher selectivity for COX-2 than COX-1 cause less gastro-intestinal toxicity than non-selective NSAIDs (Thomson and Hunt, 2012).

Examples of non-selective NSAIDs include: Ibuprofen, naproxane, indomethacin, aspirin, diclofenac, ketoprofen, piroxicam among others while selective NSAIDs include: celecoxib, rofecoxib, etoricoxib, valdecoxib, parecoxib and lumiracoxib (Thomson and Hunt, 2012).

2.4.3.3 Zollinger- Ellison Syndrome

Zollinger-Ellison Syndrome is a rare disorder that occurs when gastrinomas (tumours producing gastrin hormone) in the pancreas and duodenum stimulate gastric acid secretion. The gastrinomas, release large amounts of gastrin that cause the stomach to produce large amounts of acid. This syndrome is suspected in patients with ulcers who are not infected with *H. pylori* and who have no history of NSAIDs use. Diagnosis is confirmed by measurement of serum gastrin hormone levels (Morsy and El-sheikh, 2011).

2.4.3.4 Gastro-oesophageal reflux disease

Gastro-oesophageal reflux disease, heart burn or dyspepsia are terms used for acid reflux disease (Ahmed *et al.*, 2012). It is defined as mucosal damage produced by the abnormal reflux of gastric contents into the oesophagus (De Vault and Castell, 2005). It can also be defined as a burning pain behind the breast bone which moves upwards towards the throat. The disease can be extremely painful and make breathing difficult. Symptoms of acid reflux disease can occur at any age. Pregnancy, heavy meals, excessive weight, lack of sleep can all contribute to the disease. Symptoms include heartburn and acid regurgitation,

nausea, epigastric discomfort, dysphagia, chest pain, flatulence and cough (Ahmed *et al.*, 2012).

2.4.3.5 Oxidative stress

Oxidative stress is caused by an imbalance between production of reactive oxygen and the biological system to readily detoxify the reactive intermediates which ultimately lead to oxidative deterioration of proteins, lipids and deoxyribonucleic acid (Priya *et al.,* 2012). Reactive oxygen species or free radicals can be defined as any chemical species capable of independent existence that contain one or more unpaired electrons in their outer orbital. Oxygen is important for aerobic life processes however, a small percentage (5%) of inhaled oxygen is converted to free radicals such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (Parra-cid *et al.*, 2011). They are generated through many normal metabolic processes and are needed for normal functioning of the body. Various antioxidant enzymes like superoxide dismustase, catalase and gluthathione peroxidase and non-enzymes like Vitamin A, C and E control their accumulation. Thus an imbalance in the activity of these enzymes can lead to accumulation of the free radicals and can cause PU (Tandon *et al.,* 2004).

2.4.4 Risk factors for developing peptic ulcer disease

2.4.4.1 Smoking

Cigarette smoking causes reduction of circulating epidermal growth factor and increases free radical production in gastric mucosa (Mustafa *et al*., 2015)

2.4.4.2 Alcohol intake

Alcohol causes mucosal cell injury and depletion of gastric mucous content. Gastro-intestinal mucosal damage starts with microvascular injury which results in increase vascular permeability, oedema formation and epithelial lifting (Sunil *et al.*, 2012).

2.4.4.3 Hereditary

Individuals with type "O" blood have a higher incidence of *H. pylori* infection than do persons with other blood types. Research has shown that this is due to the presence of an antigen in type O blood which facilitates adherence of the *H. pylori* organism to the gastric mucosa (Valliani *et al*., 2013). "Molecular mimicry" occurs with *H. pylori.* It produces certain chemical components in its cell wall that resembles molecules made by the host's stomach cells. This creates a problem for the immune system. The mimicry disguises the *H. pylori* from being attacked and destroyed by the immune system. This molecule produced by *H. pylori* is identical to human blood group molecules called Lewis antigen. Edgren *et al* (2010) in their co-hort study on the risk of gastric cancers and PU in relation to ABO blood type, confirmed that PU risk was highest among those with blood group O. Also, Abdulridha, 2013 in his study on the relationship between ABO blood group distribution and the incidence of upper gastric and duodenal ulcer in Iraqi patients, observed higher incidence of DU in patients with blood group O (65.6%) compared to GU patients (54.1%). Blood group O individuals express higher inflammatory responses to *H. pylori* with higher levels of lymphocyte infiltration in the gastro-intestinal mucosa. In addition, they do not produce the substance on the surface of blood cells that may protect the lining of the duodenum. All these explain the possible cause of these individuals' increased susceptibility to PU (Abdulridha, 2013).

2.4.4.5 Stress

Stress causes the digestive tract to slow down and more gastric acid is allowed to accumulate in the stomach. Increased stomach acidity may predispose or aggravate an already present ulcer. Stress can also cause change in appetite, leading to excessive eating or lack of appetite. Excessive eating causes the stomach to produce more acid, while lack of appetite will subject the stomach mucosa to the acid produced in an empty stomach (Morsy and El-sheikh, 2011).

Others include: family history, age, gender, ethnicity and socio-economic status.

2.5 Complications of Peptic Ulcer Disease

Complications of PUD vary in frequency geographically and they include perforation, haemorrhagic ulcers, penetrating ulcers and gastric outlet obstruction.

2.5.1 Perforation

It is often the first clinical presentation of PUD. It occurs in 2–10% of patients and accounts for more than 70% of deaths associated with the disease (Bertlef and Lange, 2010). It is one of the most common complications of PUD and is more common in younger men than women (Dordevic *et al.,* 2011). The perforation site usually involves the anterior wall of the duodenum, although it might occur in antral and lesser-curvature region of the stomach (Bertleff and Lange, 2010).

2.5.2 Haemorrhagic ulcers

PU is the most common cause of acute upper gastro-intestinal bleeding accounting for about 35-50% cases of haemorrhage (Ayantunde, 2014). Bleeding ulcers are the most common complication of PUD even in children and results in high morbidity and mortality.

The incidence is more common in males than females and increases with age. The major risk factors for bleeding ulcers are: *H. pylori* infection, NSAIDs use and stress (Sotoudehmanesh *et al*., 2005).

2.5.3 Penetrating ulcers

Penetrating ulcers into organs like the liver is a rare but serious complication of PUD and may lead to unusual complications such as abscess formation or upper gastro-intestinal haemorrhage (Kayacetin and Kayacetin, 2004).

2.5.4 Gastric outlet obstruction

This refers to complete or incomplete obstruction of the distal stomach, pylorus or proximal duodenum. It may occur as a result of pyloric stenosis, mechanical obstruction due to ulcers, tumours or gastric polyps as well as acute oedema and malignancies of the stomach, pancreas and duodenum. Treatment is by surgery and it occurs in about 5% of patients (Jaka *et al*., 2013).

2.6 Diagnosis of Peptic Ulcer Disease

Various diagnostic tests for *H. pylori* have been developed and they can be broadly classified into invasive and non-invasive tests. Invasive tests require endoscopy and gastric biopsy samples. They are also expensive and require a specialist (Abiodun *et al.*, 2010; Sowande and Aldrink, 2011). The choice of a test depends on the sensitivity and specificity of the test, cost effectiveness of the test, clinical information required and local availability of the test (Khalifehgholi *et al*., 2013; Lopes *et al*., 2014).

2.6.1 Invasive tests

2.6.1.1 Rapid urease test

This test is considered the endoscopic diagnostic test of choice. It involves testing for the enzyme urease. The test is based on the principle that abundant urease produced by *H. pylori* hydrolyses urea to ammonia. The consequent rise in pH of the medium is detected by phenol red indicator (Parimala *et al*., 2014).

2.6.1.2 Histology

For proper histopathological examination, an experienced pathologist and good quality biopsies are very important. Biopsy samples should be taken from more than one site preferably the lesser curvature, antrum and corpus region. Haematoxylin and eosin (H&E), and Giemsa stains can be used to stain the biopsy samples to evaluate inflammatory cells and to detect *H. pylori* respectively (Khalifehgholi *et al.*, 2013).

2.6.1.3 Polymerase chain reaction

Here, biopsies can be taken from the antral and corpus region of the stomach and used for detection of Vag A and Cag A genes by total deoxyribonucleic acid extraction (De Martel *et al.,* 2010).

2.6.2 Non-invasive Tests

2.6.2.1 Urea breath test

This test is considered safe, cost effective, precise and easy to perform with excellent accuracy both for initial diagnosis of *H. pylori* infection and for confirmation of its eradication after treatment (Mauro *et al*., 2006). The technique involves the use of ^{14} C (radioactive isotope) or^{13} C (non- radioactive isotope) of urea to diagnose the presence of *H. pylori* in gastric and duodenal mucosa.^{13}C isotpope is preferred due to its stability hence it can be used in children, pregnant women and women of child bearing age as opposed to ^{14}C (Gisbert and Pajaras, 2004; Di Rienzo *et al*., 2013). *H. pylori* produce high

concentration of urease which is an enzyme that hydrolyzes urea into ammonium and bicarbonate. The bicarbonate generated in gastric mucosa enters the blood stream, is transported to the lungs, and is rapidly excreted from the lungs as CO_2 (Felz *et al*., 1997). Breath ID® device was approved by food and drug administration in 2001 which has the advantages of producing immediate test result, shortening the test duration with no loss of sensitivity and specificity, having a standardized test drink (4.5 g citric acid based powder and 75 mg ^{13}C-urea) and it is good for children as it does not require active cooperation (Schmilovitz-Weiss *et al.,* 2012). Four to six hours of fasting is considered before carrying out the procedure and no anti-PUD therapy is given 4 weeks prior to the procedure to prevent food from interfering with the result by delaying the excretion of CO_2 and also to prevent false negative results respectively. A breath sample should also be taken before the procedure to compare results (Di Rienzo *et al*., 2013).

2.6.2.2 Stool antigen test

This test is simple to perform and relatively cheap. It is particularly appropriate for children as stool can be obtained from them without their active collaboration as compared with samples collected by endoscopic examination (Refaay *et al*., 2006).

2.6.2.3 Antibody based test (Serology)

This is a useful non-invasive method for determining *H. pylori* eradication (Miki, 2011).

2.7 Pharmacological Management of Peptic Ulcer Disease

Many new approaches such as herbal treatment, copper complexes, nitric oxide, growth factors and probiotics are available for the treatment of PUs (Kansara and Sakhreliya, 2013).

There are different drugs used to treat PUD and this treatment depends on its cause**.** Three classes of drugs have been shown to have a direct effect on *H. pylori*: antibiotics, bismuth salts, and anti-secretary agents like H2RA and PPIs. *H. pylori* is difficult to eradicate thus most treatment regimen combine agents from two or even all three of these classes (Magaji *et al.*, 2007). For *H. pylori* induced PUD, treatment involves a PPI and two antibiotics, which may include clarithromycin, amoxicillin or metronidazole. For NSAIDs induced PUD, low dose NSAIDs with a mucosal protective drug like prostaglandin analog (misoprostol) or an anti-secretary drug like PPIs (omeprazole) or H2RA (cimetidine) can be used. For hyper secretary conditions and idiopathic ulcers, anti-secretary drugs like PPIs can be used (Bhowmik *et al.*, 2010).

2.7.1 Antacids

Most antacids contain inorganic salts like sodium bicarbonate, calcium carbonate, aluminium hydroxide and magnesium hydroxide. They act by neutralizing gastric HCl acid thus forming neutral products, reducing the flow of acid into the duodenum following a meal and inactivation of pepsin by raising the gastric pH above 4-5 (Amin, 2008). They should be taken 1 and 3 h after meal and at bed time to achieve maximum therapeutic outcome (Ganong, 2001). Antacids especially those containing aluminium and magnesium salts reduce the absorption of some antibacterials like erythromycin, ciprofloxacin, rifampicin and other drugs like ketoconazole and chloroquine (Amin, 2008).

2.7.2 Mucosal cytoprotective drugs

2.7.2.1 Prostaglandin analogues (Misoprostol)

Misoprostol inhibits gastric acid secretion and stimulates defense mechanisms. It is excreted by the kidney and the most common side effects are diarrhoea and an increase in

electrolyte and water excretion. Misoprostol causes contraction of uterine smooth muscle. The dose of misoprostol used in PUD is 800 mcg per day in two or four divided doses for 4 weeks (Eswaran and Roy, 2005). It is also used in combination with diclofenac marketed as Arthotec® by Pfizer to decrease GI complications caused by NSAIDs.

2.7.2.2 Bismuth salts

At acidic pH, bismuth salt forms a complex with mucous to coat the mucosal surface and ulcer base. This coat protects against acid and pepsin digestion. It also stimulates mucous production and may chelate with pepsin, thus speeding ulcer healing (Amin, 2008).

2.7.2.3 Sucralfates

Sucralfate is an aluminium salt of sulfated sucrose. In an acidic environment, the aluminum and sulfate dissociate to form a sulfide. This highly polar anion binds to cations, such as proteins and mucins and this forms a protective barrier and stimulates bicarbonate, mucous, and growth factor release (Sowande and Aldrink, 2011).

2.7.3 Histamin-2 receptor antagonist (H2RA)

Examples include cimetidine, ranitidine, famotidine and nizatidine. They have an aromatic ring with a flexible side chain similar to the histamine molecule. These agents are specific antagonists that inhibit acid secretion by competitively and reversibly blocking the H2R on the basolateral membrane of the parietal cell. The drugs differ slightly in structure but have many similarities in their pharmacological properties. H2RA inhibits basal and meal stimulated acid secretion. As the longest period of basal acid secretion occurs nocturnally, dosing after an evening meal or at bedtime is optimal for these agents (Huang and Hunt, 2001). They block histamine action on the H2R which plays a major role on parietal cell proton pump activation. These agents are rapidly absorbed from the intestine and undergo

first-pass metabolism. Absorption is not affected by the ingestion of food, but is decreased by antacids (Tuskey and Peura, 2013). Hepatic metabolites are excreted by the kidney therefore doses are reduced in renal impairment. All H2RA have similar side effects. They cause tachyphylaxis with chronic use, myelosuppression, leucopenia and neutropenia. Anaemia and thrombocytopenia have been associated with all H2RA and central nervous system side effects such as headache, lethargy, dizziness, depression, memory loss, agitation, confusion, psychosis and hallucinations can occur. Cimetidine, is an anti-androgenic drug causing gynecomastia and impotence in males and galactorrhea in females. Cimetidine and to a lesser extent ranitidine are cytochrome P450 hepatic enzyme inhibitors. They bind to this enzyme and inhibit the elimination of drugs metabolised by the enzyme leading to toxic blood levels of these drugs (Hoogerwerf and Pasricha, 2006). Fixed dose combination therapy of NSAIDs and H2RA has been shown to reduce NSAID induced PU. Example is 800 mg ibuprofen+26.6 mg famotidine (Tuskey and Peura, 2013).

2.7.4 Proton pump inhibitors (PPIs)

Examples include omeprazole, esomeprazole, rabeprazole, lansoprazole and pantoprazole. They are potent irreversible inhibitors of the H^+-K^+ATPase pump. This enzyme is located in the apical secretory membrane of the parietal cell and plays a key role in the secretion of H^+ (Huang and Hunt, 2001). PPIs are considered prodrugs which are reabsorbed in the small intestine then transported to the parietal cells in the stomach to be protonated into the active form. They bind covalently and predominantly to those proton pumps that are actively secreting acid thus they are most effective if administered immediately before meals. The absorption of lansoprazole, pantoprazole, and rabeprazole is delayed by food, but not affected by antacids. PPIs are metabolized almost completely by the hepatic

cytochrome P450 enzyme hence reducing the clearance of drugs like warfarin, phenytoin and diazepam and metabolites are eliminated by the kidney. There is no need for dose adjustment in hepatic or renal disease. Once-daily dosing in the morning is more effective than dosing in the evening for all PPIs with respect to the suppression of intragastric acidity and daytime gastric acid secretion in particular, which may result from a better bio-availability being achieved with the morning dose. When higher doses are needed, these drugs must be given twice daily to achieve the optimal suppression of 24 hour intragastric acidity (Huang and Hunt, 2001). Tolerance or tachyphylaxis is not seen as with the H2RA. The most common side effects are headaches, diarrhea, abdominal pain, constipation, and nausea. Drug interaction usually occurs with drugs that require stomach acid for absorption like ketoconazole and ampicillin hence if a PPI and antifungal agent is required for therapy, an agent other than ketoconazole should be chosen. On the other hand, a drug like digoxin requires an elevated gastric pH for absorption so when given with PPIs, plasma digoxin levels should be monitored (Eswaran and Roy, 2005). A fixed dose combination therapy of naproxen an NSAID and esomeprazole a PPI exist to decrease the risk of GI complications caused by NSAIDs (Tusky and Peura, 2013).

2.8 Treatment of Peptic Ulcer Disease caused by *Helicobacter pylori*

2.8.1 First-line therapy

2.8.1.1 Standard triple therapy

This involves the use of one PPI and two antibiotics for 10-14 days or as long as 4-8 weeks as the case may be.

For example: clarithromycin 500 mg twice a day plus amoxicillin 1g twice a day and a PPI (omeprazole 20 mg twice a day) (Lee *et al*., 2015).

2.8.1.2 Sequential therapy

This is currently recommended as an alternative first-line treatment for *H. pylori* infection due to treatment failure of standard triple therapy caused by antibiotic resistance especially to clarithromycin and metronidazole (Chang *et al*., 2017). It involves four drug combinations for 10- 14 days.

Examples

Clarithromycin-based sequential therapy: 40 mg of pantoprazole or 20 mg of rabeprazole plus 1 g of amoxicillin twice a day for the initial 5-7 days, followed by 40 mg of pantoprazole or 20 mg of rabeprazole and 500 mg of clarithromycin twice a day and 500 mg of metronidazole three times a day for the subsequent 5-7 days (Hwang *et al*., 2015; Lee *et al*., 2016).

Moxifloxacin-based sequential therapy: 20 mg rabeprazole and 1 g amoxicillin twice daily for the first week followed by 20 mg rabeprazole twice daily, 500 mg metronidazole twice daily and moxifloxacin 400 mg once daily for the remaining week (Hwang *et al*., 2015).

2.8.2 Second-line therapy

2.8.2.1 Bismuth containing quadruple therapy

This consists of bismuth subsalicylate 525 mg four times a day plus metronidazole 250 mg four times daily, tetracycline 500 mg four times daily with a PPI twice daily for 14 days or H2RA for 28 days (Ramakrishnan, 2007). A modified quadruple therapy consists of bismuth subcitrate 600 mg, tetracycline 1 g, amoxicillin 1 g and pantoprazole 40 mg or any PPI twice daily (Lee *et al*., 2016).

2.8.2.2 Levofloxacin-based therapy

This consists of amoxicillin 1 g, levofloxacin 200 mg and omeprazole twice daily for 7-14 days (Lee *et al*., 2015).

2.8.2.3 Moxifloxacin-based therapy

This consists of moxifloxacin 400 mg four times a day plus amoxicillin 1 g twice daily and omeprazole twice daily for 7-14 days (Lee *et al*., 2015).

Other antibiotics that can be used in case of resistance include: Rifabutin, furazolidone, tinidazole and tetracycline (Di Mario *et al*., 2006).

2.8.3 Vaccines

Therapeutic vaccination is a good strategy to protect against *H. pylori* infection. *H. pylori* has several virulence factors some of which have the potential of being used as vaccines (Kabir, 2007). A multi epitome vaccine called BIB was constructed by linking cholera toxin B subunit with different sub units of urease I and urease B to form a recombinant vaccine which was tested orally in BALB/c mice and found to be protective against *H. pylori* (Yang *et al*., 2015).

2.9 Antimicrobial Resistance

Emergence of antimicrobial resistant *H. pylori* is an important challenge in the treatment of PUD. Some studies indicate worrisome levels of resistance to metronidazole and clarithromycin used to treat PUD. Resistance to either metronidazole or clarithromycin has been associated with markedly reduced eradication rates after extended treatment with multi-agent regimens. Second generation floroquinolones like levofloxacin and moxifloxacin have proved to have high *H.pylori* eradication rates of about 90% in first-line treatments and over 70% in second line treatments (Zhang *et al*., 2013). It is mandatory for

patients who do not respond to second line therapy, to carry out antimicrobial susceptibility test (Fischbach *et al*., 2009).

2.10 The Plant *Waltheria indica* (Linn)

W. indica belongs to the family Sterculiaceae which is a family of tropical and sub-tropical plants comprising of nearly 60 species (Saunders, 2007). It is indigenous to Hawaii, Mexico and West Africa but can be found in many other parts of the world (Zailani *et al.*, 2010).

2.10.1 Plant taxonomy

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Malvales

Family: Sterculiaceae

Genus: *Waltheria*

Species: *Waltheria indica*

2.10.2 Plant Description

It is an erect perennial shrub that grows up to 500 mm high. Leaves are alternate with serrated edges and acute tip. Its flowers are yellow; occur in clusters and flowers in about six months. The roots are brown and flexible with a strong stem emerging from the ground. Globally, its distribution and habitat is mostly in subtropical and tropical zones (Saunders, 2007).

W. indica is also known as ‘sleepy morning’, ‘velvet leaf’, ‘marsh- mallow’, ‘monkey bush’, ‘boater bush’, ‘ leather coat’, ‘buff coat’ (Burkill, 2000). In Hawaii, it is called ‘ Uhaloa’, in Portuguese ‘mato-branco’ , in Senegal ‘fulutalut’ or ‘fulutafu’ , in Hausa ‘hankufa’ or ‘yankufa’ , in Fulfulde ‘kafaffi’, in Yoruba ‘Ewe-epo’ or ‘korikodi’ (Burkill, 2000).

2.10.3 Ethnomedicinal uses of the plant

Different parts of the plant are used in different countries of the world for the treatment of various ailments and within a country, different regions use different parts of the plants as drug. The root is used to treat stomach aches, diarrhoea, dysentery, haemorrhoids, intestinal haemorrhage, skin disorders, fever, pain, inflammation, epilepsy, cough, sore throat, syphilis, infertility, alopecia, night blindness and as an aphrodisiac and contraceptive. The leaves are used to treat gastric ulcer, diarrhoea, inflammation, conjunctivitis, convulsion, gingivitis, anaemia, syphilis and rheumatism. The whole plant is used to treat diarrhoea, dysentery and asthma (Zongo *et al*., 2013).

In Hawaii, *W. indica* is used to treat leprosy, inflammation, neuralgia, sore throat, asthma and as a laxative. In Mexico, it is used to treat wounds, skin ulcers, rheumatism, stomach aches, diarrhoea and dysentery. In India, the root-bark and flowers are used to treat oral thrush and the root is used as anti-rabbis (Burkill, 2000). In Nigeria, the root is chewed or a hot decoction taken morning and evening as cough medicine. It is also used to treat intestinal pain, diarrhoea, infertility, skin diseases and gonorrhoea (Zailani *et al.*, 2010).



Plate I: *Waltheria indica* in its natural Habitat at area BZ, Ahmadu Bello University Staff Quarters, Samaru-Zaria (snapped by Ngyama, January, 2014)



Plate II: Whole plant of *Waltheria indica* showing leaves, flowers and stem (snapped by Ngyama, July, 2017)



Plate III: Root of *Waltheria indica* after harvest (snapped by Ngyama, January, 2014)

2.10.4 Previous scientific studies on *Waltheria indica*

Some previous studies carried out on the plant include: Evaluation of its haematinic potential by Oladiji *et al*., 2005 and Basiru and Olayemi, 2014, study on its analgesic activity by Mohammed *et al*., 2007, evaluation of its sedative and anticonvulsant activity in mice by Hamidu *et al*., 2008, determination of its antimicrobial activity (Zailani *et al*., 2010; Bala *et al*., 2011 and Olajuyibe *et al*., 2011) as well as determination of its antioxidant and antimicrobial activity (Garba *et al*., 2012; Mongalo *et al*., 2012). Study on its ethno-pharmacological uses and phytochemical Screening by Kone *et al*., 2013 and its biological activity by Raga *et al*., 2013. Study on its botany, traditional uses, phytochemistry and pharmacology by Zongo *et al*., 2013 and evaluation of its anti-cataract potential by Atif *et al*., 2014. Study on the phytochemical constituents, invitro antioxidant and cytotoxic activity (Krishna, 2015), the evaluation of its aphrodisiac activity (Baskaran and Ranjani, 2016), evaluation of its anti-ulcer activity against pyloric ligated ulcer model in rats (Jacob and Srinivasan, 2016), study on its antidiabetic and antioxidant properties (Kannan *et al*., 2016), study on its phytochemical and antimicrobial activities (Musa *et al*., 2016) evaluation of its antioxidant, analgesic and anti-inflammatory activities (Yougbare-Ziebrou *et al*., 2016), evaluation of its hepatoprotective effect against various NSAIDs-induced hepatic damage in rats (Chandekar *et al*., 2017) GC-MS analysis of its bioactive compounds and their pharmacological activities (Banakar and Jayaraj, 2017) and finally, standardization and evaluation of its anticonvulsant activities (Igwebuike *et al*., 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Drugs and Chemicals (Analar grade)

Absolute methanol (BDH Poole, England)

Acetic acid anhydride (BDH chemicals Ltd Poole, England)

Ammonia (Johnson Solomon Ltd London, England**)**

Calcium chloride (Hopkin and Williams)

Chloroform (LOBA Chemie, India)

Cimetidine 400 mg (Smithkline and French Labs Ltd, England)

Distilled water

D-glucose (BDH chemicals Ltd poole, England)

Ferric chloride anhydrous (Labtech chemicals, Australia)

Formaldehyde (May & Baker Ltd, England)

Glacial acetic acid (May & Baker Ltd, England)

Histamine (Sigma-Aldrich Life Science, St. Louis U.S.A)

Indomethacin (Sigma-Aldrich Life Science, St. Louis U.S.A)

Magnesium chloride (Fisher Scientific Company, New Jersey)

Normal saline (Dana, Nigeria)

Omeprazole 20 mg (Hovid, Malaysia)

Phenolphthalein indicator (Merck chemicals, U.S.A)

Potassium chloride (New India chemical enterprises, Kochi)

Sodium chloride (BDH Chemicals Ltd Poole, England)

Sodium dihydrogen phosphate (Prolabo)

Sodium hydrogen carbonate (BDH chemicals Ltd Poole, England)

Sodium hydroxide pellets (BDH chemicals Ltd Poole, England)

3.1.2 Equipment

Animal cages

Starvation cages

Water bottles

Test tubes

Test tube rack

Disposable gloves

Organ bath

Scissors

Forceps

Syringes and needles (1 ml, 2 ml, 5 ml, 10 ml, 20 ml) Changzhou Yuekang Medical appliance Co. Ltd, China

DOA-PI33A-BN Oxygenator pump Benton Harbor, USA

19/00052/39 Thermocirculator Church hill Instrument Co. Ltd, England

Mettler P162 weighing balance, Greifensee- Zurich

BTL bench Centrifuge, England

Techmel and Techmel, TT-645 Centrifuge, UK

Olympus compound microscope X52-107BN X100 oil immersion lens

Hettich microhaematocrit centrifuge machine Tuttlingen D-7200

Marienfeld haemacytometer 0630010

Ugo Basile micro dynamometer model 7050

GG-17 separating funnel

50 ml beaker

50ml burette

Burette stand and clamp

30 cm rule

No. 1 Whatsmans filter paper

Needle and thread

Magnifying glass

3.2 Methodology

3.2.1 Plant collection and authentication

Waltheria indica was collected from its natural habitat at Area BZ, Ahmadu Bello University Staff Quarters, Samaru-Zaria Kaduna State, Nigeria. It was identified and authenticated in the Herbarium section of the Faculty of Life Sciences, Department of Botany Ahmadu Bello University, Zaria by the taxonomist and it has a reference voucher specimen number 600.

3.2.2 Extraction of the root of *Waltheria indica*

The root was removed from the whole plant, washed under running water and air dried under shade until a constant weight was obtained. It was then crushed into powder with pestle and mortar and 700 g of the powder was cold macerated with 2.5 litres of 70% v/v methanol for 72 h in a separating funnel clogged with cotton wool. The separating funnel was shaken intermittently and the content filtered using no 1 whatsman's filter paper. The filtrate was poured into an evaporating dish and concentrated to dryness over a water bath at 40-50ºC which yielded 16.7% w/w methanol extract. This extraction was carried out in the Laboratory of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria.

3.2.3 Experimental animals

Adult apparently healthy Wistar rats of both sexes, guinea pigs and rabbits were obtained from the animal house of the Department of Pharmacology and Therapeutics Ahmadu Bello University, Zaria and were maintained under laboratory conditions with food and water provided. The animals were allowed free access to standard rodent feed and water *ad libitum* prior to the experiment. The experimental protocol was approved by the Ahmadu Bello University Committee on Animal use and Care (ABUCAUC) with a certificate number of ABUCAUC/2018/022.

3.2.4 Preliminary phytochemical screening

The preliminary phytochemical screening was carried out by the methods described by Trease and Evans, 2002 and as summarized below.

3.2.4.1 Test for alkaloids

Meyer's Test: To a portion of the extract, few drops of Meyer's reagent was added. A cream precipitate indicated presence of alkaloids.

Dragendoff's Test: To a portion of the extract, few drops of Dragendoff reagent were added. A reddish brown precipitate indicated presence of alkaloids.

Wagners Test: Few drops of Wagners reagent was added to a portion of the extract and a whitish precipitate indicated presence of alkaloids.

3.2.4.2 Test for cardiac glycoside

Keller-kiliani Test-A portion of the extract was dissolved in 1 ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1 ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. A pale green colour in the upper acetic layer indicated the presence of cardiac glycosides.

3.2.4.3 Test for flavonoids

Sodium hydroxide Test- A few drops of 10% sodium hydroxide was added to the extract in a test tube and yellow colouration indicated presence of flavonoids.

3.2.4.4 Test for free anthracene derivatives

Bontrager's Test-To a portion of the extract in a dry test tube, 5 ml of chloroform was added and was shaken for at least 5 min. This was filtered and the filterate shaken with equal volume of 10% ammonia solution. Bright pink colour in the aqueous (upper)layer would indicate the presence of free anthraquinones.

3.2.4.5 Test for saponin glycoside

Frothing test-About10 ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 sec. The tube was allowed to stand in a vertical position and was observed for 30 min. A honeycomb froth that persisted for 10-15 min indicated presence of saponins.

3.2.4.6 Test for tannins

Ferric Chloride Test-To a portion of the extract,3-5 drops of ferric chloride solution was added. A greenish black precipitate indicated presence of condensed tannins.

3.2.4.7 Test for unsaturated steroid and triterpene

Liebermann-Bucchard Test-To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. One millilitre of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immediately and over a period of 1 h. Blue to blue green colour in the upper layer and a reddish pink or purple colour would indicate the presence of triterpene.

3.3 Toxicological Studies

3.3.1 Acute toxicity study in Wistar rats

The acute toxicity study was carried out using modified Lorke's (1983) method and using rats of both sexes. The rats (150-180 g) were fasted overnight and the median lethal dose (LD_{50}) evaluation was carried out in two phases. In the first phase, nine rats were randomly divided into three groups of three rats each. Group 1, 2 and 3 were administered 10, 100 and 1,000 mg/kg body weight p.o of the extract respectively. The rats were observed for signs of toxicity and death within 24 h. Since no death occurred in the first phase, in the

second phase, three rats were divided into three groups of one rat in each group. Group 1, 2 and 3 were administered 1,600, 2,900 and 5,000 mg/kg body weight p.o of the extract respectively. The rats were also observed for signs of toxicity and death within 24 h. Since no death occurred, the LD_{50} was estimated to be greater than 5,000 mg/kg.

3.3.2 Sub-acute toxicity study in Wistar rats

The repeated dose 28-day oral toxicity study in Wistar rats was carried out in accordance with OECD 407, (2008) guidelines. Twenty four Wistar rats of both sexes (170-210g) were randomly divided into four groups of six rats each. Rats in group 1 which served as control were administered 1ml/kg body weight of distilled water p.o while rats in groups 2, 3 and 4 were administered 250, 500 and 1,000 mg/kg body weight of MEWI p.o respectively once daily for 28 days. The rats had free access to food and water ad libitum throughout the duration of the study and were observed daily for signs of toxicity and mortality. The rats were weighed every week at 9 am using a sensitive balance before the commencement of dosing and on the last day of the study. The body weight of each rat was recorded. On the 29^{th} day, the animals were sacrificed in a chloroform chamber and blood samples were collected from the jugular vein. One portion was collected into K^{+}EDTA (potassium ethylenediaminetetraaceticacid) bottles for haematological studies such as estimation of packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell count (RBC), platelet, white blood cell count (WBC) and differentials using Hettich microhaematocrit centrifuge. This study was carried out in the pathology Laboratory of Faculty of Veterinary Medicine, A.B.U. Zaria. The other portion was dispensed into plain bottles allowed to clot and centrifuged at 805 g. The serum was separated, stored at -8 to 2°C and used for biochemical studies: alanine transaminase (ALT), aspartate transaminase (AST) and

alkaline phosphatase (ALP) levels as well as albumin, total protein, total and conjugated bilirubin, urea and creatinine. Electrolytes like sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) were also evaluated. This study was carried out at the National Eye Centre Laboratory, Kaduna. Different organs namely the stomach, intestine, liver, kidney and heart were removed from the rats and weighed. The relative organ body weight ratio (ROW) of each rat was calculated and the organs removed were stored in 10% formalin and taken for histology studies at the Department of Anatomy, A.B.U. Zaria.

3.4 Gastro-intestinal Mucosal Protective Studies

3.4.1 Indomethacin-induced ulcer in Wistar rats

The indomethacin induced ulcer study was carried out by the method described by Djahanguiri (1969). Thirty Wistar rats of both sexes (140-180 g) were divided into five groups of six rats each. The rats were fasted in starvation cages by deprivation of food for 48 h before the experiment to ensure complete emptying of the stomach while water was allowed *ad libitum*. The rats were weighed after the fasting period and rats in group 1 were administered 1 ml/kg of distilled water (negative control) orally while those in groups 2, 3 and 4 were administered extract doses of 250, 500 and 1,000 mg/kg orally respectively. Rats in group 5 (positive control) were administered 100 mg/kg cimetidine orally. After 40 min, oral indomethacin in a dose of 20 mg/kg per body weight was administered to the rats in each of the groups. Six hours later, the rats were anaesthesized in a chloroform chamber and the abdomen of each rat was opened by a mid-line incision to remove the stomach into 2% v/v formal saline. The overnight stored stomachs were opened up along the greater curvature the next day and washed under running water. Each rat stomach was wide spread on No. 1 Whatsman's filter paper and examined for the presence of lesions with a hand

lens. The ulcerated surfaces in each stomach were measured with a transparent millimetre scale rule and the mean ± S.E.M ulcer surfaces in each group were calculated and expressed as ulcer index (U.I). The Preventive index % (P.I. %) was then calculated as

$$\text{P.I. \%} = \frac{U.I.Indomethacin - U.I.Extract}{U.I.Indomethacin}\ x\ 100$$

3.4.2 Pylorus ligation-induced ulcer in Wistar rats

The Pylorus ligation-induced ulcer study, was carried out by the method described by Shay *et al.,* 1945.

3.4.2.1 Administration of water, extract and omeprazole

Forty Wistar rats of both sexes (150-200 g) were divided into five groups of eight rats each. The rats were fasted in starvation cages for 48 h to ensure complete emptying of the stomach but had access to drinking water *ad libitum*. The animals were weighed after the fasting period and rats in group 1 were administered 1 ml/kg of distilled water p.o while those in groups 2, 3 and 4 were administered extract doses of 250, 500 and 1,000 mg/kg p.o. Rats in group five were administered 20 mg/kg omeprazole p.o. 1 h later, the rats were mildly anaesthesized in a chloroform chamber and a mid-line abdominal incision made on each rat using a blade. The pylorus was located and ligated with a cotton thread. The abdominal wall was sutured up and cleaned thoroughly with normal saline and the animals left for 19 h for accumulation of gastric juice. All the rats were again anaesthesized in a chloroform chamber and the abdomen opened up to remove the stomachs. The content of each stomach was drained into a centrifuge tube and centrifuged at 313 g to collect the supernatant while the stomach of each rat was washed and then spread out on a filter paper

and examined with a hand lens for the number of ulcers. The severity of ulcer lesions was scored as described by Desai *et al*., 1999.

0 No ulcer

1 Superficial mucosal erosion

2 Deep ulcer

3 Perforated or penetrated ulcer

The Ulcer index (U.I) was calculated as

$UI= UN+US+UPX10^{-1}$

UN=Average number of ulcers per animal

US=Average of severity Score

UP=Percentage of animals with ulcer

For the average number of ulcers per animal, the number of ulcers on each rat's stomach was counted and the average for each group calculated.

For the average of severity score, the severity score of each rat's stomach was added and the average calculated for each group.

For the percentage of animals with ulcer, the number of rats with ulcerated stomach was counted and divided by the number of rats in each group multiplied by 100.

3.4.2.2 Analysis of the gastric content

After the centrifugation, the supernatant was measured with a measuring cylinder and the total volume of the supernatant for each group was recorded as the gastric content volume.

3.4.2.3 Quantitative determination of the titritable acidity:

The titratable acidity of the gastric content of each group was then determined using 1 ml of the gastric content mixed with 2 drops of phenolphthalein indicator in a conical flask and titrated against 0.1 N NaOH. The volume of 0.1 N NaOH at which the colour change was observed was then taken as the titratable acidity expressed as milliequivalents per litre (mEq/L). It is defined as the volume in ml of 0.1 N NaOH required to neutralize 100 ml of the gastric content (Davenport, 1982).

$$\text{Titritable acidity (mEq/L)} = \frac{\textit{Volume of NaOH} \times N}{0.1} \times 100$$

Where N= Normality of NaOH

This was therefore calculated for each group and compared.

3.5 Isolated Tissue Experiment

3.5.1 Isolated guinea pig ileum

Adult guinea pig was sacrificed humanely after fasting for 24 h and the abdomen opened. The ileum was removed from the freshly killed animal and segments (about 3 cm) of the tissue were cut and placed in a petri-dish containing Tyrode solution. The isolated tissues were suspended vertically in a 10 ml organ bath containing aerated freshly prepared Tyrode solution at 37^{O}C. The tissues were set up under a tension of 1 g and responses were recorded on a microdynamometre. At the end of 30 min equilibration period, the responses of the tissues to different concentrations of histamine (to obtain a standard concentration-response curve) and different concentrations of the extract (1 mg/ml, 10 mg/ml and 100 mg/ml) were established. Contact time for histamine and MEWI with the tissue was 1 min after which the tissue was washed three times using Tyrodes solution. A resting period of 5

min was allowed between histamine and extract additions and the final organ bath concentration (FOBC) of histamine and the different doses of MEWI was calculated after the experiment.

3.5.2 Isolated rabbit jejunum

The method was the same as that of isolated guinea pig ileum except that in this case, adult rabbit jejunum was used and the effects of graded doses of acetylcholine (Ach) and the extract were evaluated and responses recorded on a microdynanometre. The FOBC of Ach and the different doses of MEWI were calculated after the experiment.

3.6 Statistical Analysis

Results were expressed as mean ± SEM (standard error of mean) and presented as tables, plates and figures. Data was analysed using SPSS version 20 and subjected to one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Results were regarded as significant at $p \leq 0.05$

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield

Cold macerating 700 g of powdered *W. indica* root in 70% v/v methanol gave an extract weight of 116.75g and percentage yield of 16.7% w/w.

4.2 Preliminary Phytochemical Screening of Methanol Root Extract of *Waltheria indica*

In the preliminary phytochemical screening of MEWI, only free anthracene and unsaturated steroids were absent. Other phytochemical constituents such as alkaloids, cardiac glycosides, flavonoids, saponin glycosides, tannins and triterpenes were present.

Table 4.1 Preliminary phytochemical constituents in methanol root extract of *Waltheria indica*

Constituents	Remarks
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Free anthracene	–
Saponin glycoside	+
Tannins	+
Triterpenes	+
Unsaturated steroids	–

Key

\+ = Present

\- = Absent

4.3 Acute Toxicity Study

The oral LD_{50} of the extract was estimated to be greater than 5,000 mg/kg body weight in Wistar rats.

4.4 Sub-acute Toxicity Study

4.4.1 The effect of methanol root extract of *Waltheria indica* on weekly body weights of Wistar rats

There was no statistical significant difference at $p \leq 0.05$ in the body weights of the rats administered increasing doses of the extract after 28 days when compared with the control (Table 4.2).

4.4.2 The effect of methanol root extract of *Waltheria indica* on relative organ weight ratio of Wistar rats

There was no statistical significant difference at $p \leq 0.05$ in the relative organ weight of the stomach, intestine, liver, kidney and heart at all the dose points of MEWI after 28 days oral administration when compared with the control (Table 4.3).

4.4.3 The effect of methanol root extract of *Waltheria indica* on some haematological parameters in Wistar rats

The daily administration of MEWI for 28 days did not produce any statistical significant change in the haematological parameters analysed: PCV, Hb, RBC, platelets, WBC, neutrophil, lymphocyte, monocyte and eosinophil (Table 4.4).

4.4.4 The effect of methanol root extract of *Waltheria indica* on some liver function biomarkers in Wistar rats

There was statistical significant increase in ALT ($p \leq 0.01$) at 250 mg/kg MEWI. Statistical significant increase was also seen in T.BIL levels ($p \leq 0.001$) at 250, 500 and 1,000 mg/kg MEWI as well as significant increase in con.Bil levels of 1,000 mg/kg MEWI at $p \leq 0.05$ (Table 4.5).

4.4.5 The effect of methanol root extract of *Waltheria indica* on some serum electrolytes and renal function indices in Wistar rats

Although there was slight increase and decrease in serum electrolytes, urea and creatinine, it was not significantly different from control at $p \leq 0.05$ (Table 4.6).

Table 4.2: Effect of oral administration of methanol root extract of *Waltheria indica* on weekly body weight in Wistar rats within 28 days

Treatment group	Week 0 (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
D.W. (1 ml/kg)	199.17±0.38	218.83±1.29	222.50±1.78	222.50±1.07	226.50±0.56
MEWI (250 mg/kg)	202.33±1.91	213.17±0.60	219.33±1.71	219.33±0.94	220.33±1.14
MEWI (500 mg/kg)	222.00±0.69	220.33±1.51	217.67±1.35	220.00±0.57	225.67±2.44
MEWI (1,000 mg/kg)	214.75±2.70	208.75±1.49	228.00±1.40	220.00±1.02	219.75±2.01

Data is expressed as Mean ± SEM, n=6,
Statistical tool: one way ANOVA+ Dunnett's post hoc test
D.W. = distilled water
MEWI= methanol root extract of *Waltheria indica*

Table 4.3: Effect of oral administration of methanol root extract of *Waltheria indica* on relative organ weight ratio of some rat organs after 28 days

Treatment group	Stomach (g)	Intestine (g)	Liver (g)	Kidney (g)	Heart (g)
D.W. (1 ml/kg)	0.67±0.07	0.27±0.03	3.16±0.23	0.57±0.04	0.38±0.03
MEWI (250 mg/kg)	0.65±0.08	0.21±0.03	3.55±0.09	0.58±0.03	0.42±0.05
MEWI (500 mg/kg)	0.91±0.17	0.21±0.01	3.48±0.08	0.65±0.05	0.38±0.03
MEWI (1,000 mg/kg)	0.75±0.12	0.33±0.03	3.56±0.35	0.66±0.10	0.35±0.02

Data is expressed as Mean ± SEM, n = 6
Statistical tool: one way ANOVA+ Dunnett's post hoc test
D.W. = distilled water
MEWI = methanol root extract of *Waltheria indica*

Table 4.4: Effect of oral administration of methanol root extract of *Waltheria indica* on some haematological parameters in Wistar rats after 28 days

Treatment group	PCV %	Hb g/dl	RBC $x10^6/\mu L$	Platelets $x10^9/L$	WBC $x10^9/L$	Neutrophil %	Lymphocyte %	Monocyte %	Eosinophil %
D.W. (1 ml/kg)	35.33±1.82	11.75±0.61	5.80±0.36	184.17±1.19	5.63±0.58	23.17±3.84	73.33±4.30	2.00±0.00	1.50±0.5
MEWI (250mg/kg)	33.83±2.30	11.25±0.76	5.50±0.39	180.33±2.32	5.08±0.69	22.17±2.32	73.00±2.31	1.67±0.33	2.00±0.00
MEWI (500mg/kg)	33.33±3.38	11.07±1.14	5.53±0.55	185.33±1.96	3.80±0.53	19.33±1.76	77.33±2.67	1.00±0.00	0.00±0.00
MEWI (1,000 mg/kg)	35.50±3.28	11.83±1.09	5.90±0.56	183.00±0.63	3.73±0.32	16.50±3.38	80.75±2.84	1.50±0.50	0.00±0.00

Data is expressed as Mean ± SEM, n = 6
Statistical tool: one way ANOVA + Dunnett's post hoc test
D.W. = distilled water
MEWI = methanol root extract of *Waltheria indica*

Table 4.5: Effect of oral administration of methanol root extract of *Waltheria indica* on liver function biomarkers in Wistar rats after 28 days

Treatment group	ALT µ/L	AST µ/L	ALP µ/L	ALB µ/L	TP g/dL	T.BIL mmol/L	Con.Bil mmol/L
D.W. (1 ml/kg)	31.5± 2.09	38.83± 0.48	46.50± 5.81	48.67± 0.42	60.5± 6.60	14.93± 0.22	3.78± 0.08
MEWI (250 mg/kg)	69.67±0.53**	40.33±1.76	57.67± 1.75	48.33±0.21	62.50±6.23	16.03±0.19***	3.98±0.13
MEWI (500 mg/kg)	58.00±0.58	37.00±1.16	42.00±2.00	47.00±1.00	63.33±1.33	16.53±0.15***	4.03±0.15
MEWI (1,000 mg/kg)	34.75±1.79	41.00±2.38	55.25±3.30	46.00±2.71	72.50±0.65	17.03±0.10***	4.30±0.07*

Data is expressed as mean ± SEM, n=6,
Statistical tool= one way ANOVA+ Dunnett's post-hoc test
*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ (Significant difference compared with distilled water)
D.W. = distilled water
MEWI= methanol root extract of *Waltheria indica*
ALT= alanine aminotransferase, AST= aspartate aminotransferase ALP= alkaline phosphatase, ALB= albumin, TP= total protein, T.BIL= total bilirubin, Con. Bil= conjugated bilirubin

Table 4.6: Effect of oral administration of methanol root extract of *Waltheria indica* on some serum electrolytes and renal function indices in Wistar rats after 28 days

Treatment group	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L	HCO_3^- mmol/L	Urea mmol/L	Creatinine mmol/L
D.W. (1 ml/kg)	138.5± 2.59	3.42 ± 0.14	116.83± 1.86	26.67±1.63	2.52± 0.15	77± 3.81
MEWI (250 mg/kg)	140.17± 2.98	4.13± 0.70	100.83± 3.69	30.00± 1.37	3.88±1.10	67.83±2.48
MEWI (500 mg/kg)	129.67±0.88	3.67±0.35	89.00±2.08	25.67±0.33	3.13±0.07	82.67±1.33
MEWI (1,000 mg/kg)	138.50±2.60	3.95±0.76	99.00±3.49	31.25±1.75	3.20±1.10	63.00±1.07

Data is expressed as Mean ± SEM, n =6,
Statistical tool: one way ANOVA + Dunnett post-hoc test.
D.W. = distilled water
MEWI= methanol root extract of *Waltheria indica*
Na^+= Sodium ion, K^+= Potassium ion, Cl^-= Chloride ion, HCO_3^-= Bicarbonate ion,

4.5 The Histology Effect of Increasing Doses of Methanol Root Extract of *Waltheria indica* on some Wistar Rats Organs

4.5.1 The stomach

The group that received 1 ml/kg distilled water showed normal mucosa with glandular cells (Plate IV A). The group that received 250 mg/kg extract showed normal mucosa and sub-mucosa with glandular cells (Plate IV B) and the group that received 500 mg/kg extract showed normal mucosa, sub mucosa and muscularis with glandular cells (Plate IV C) while the group that received 1,000 mg/kg extract showed normal mucosa and sub mucosa with glandular cells (Plate IV D). There was no obvious histology change in the stomach mucosa of the rats after treatment with all the dose points of MEWI (Plate IV A, B, C and D).

4.5.2 The intestine

Normal intestinal architecture was seen in the rats administered 1 ml/kg distilled water (Plate V A). The rats administered 250 mg/kg extract showed normal absorptive finger like projections (Plate V B). Also, those administered 500 and 1,000 mg/kg extract showed normal absorptive glands, normal intestinal architecture with no obvious histology change (Plate V C and V D) respectively. The Wistar rats treated with the different doses of MEWI had normal intestinal features (Plate V A, B, C and D).

4.5.3 The liver

The liver of Wistar rats administered 1 ml/kg distilled water showed normal hepatocytes (Plate VI A). The 250 mg/kg extract group showed polymorphonuclear infiltration (Plate VI B) while rats treated with 500 mg/kg MEWI showed moderate hepatocellular necrosis (Plate VI C) and those treated with 1,000 mg/kg MEWI showed polymorphonuclear infiltration (Plate VI D).

4.5.4 The kidney

The kidney of Wistar rats administered 1 ml/kg distilled water, 250 and 500 mg/kg extract showed normal glomeruli and renal tubular structures (plate VII A, B, C) but tubular necrosis was seen in the kidney of rats administered 1,000 mg/kg (Plate VII D).

4.5.5 The heart

There was no obvious histology change in the myocardium of Wistar rats administered all the dose points of the extract. They all had normal cardiac cells and muscles (Plate VIII A, B, C, D).

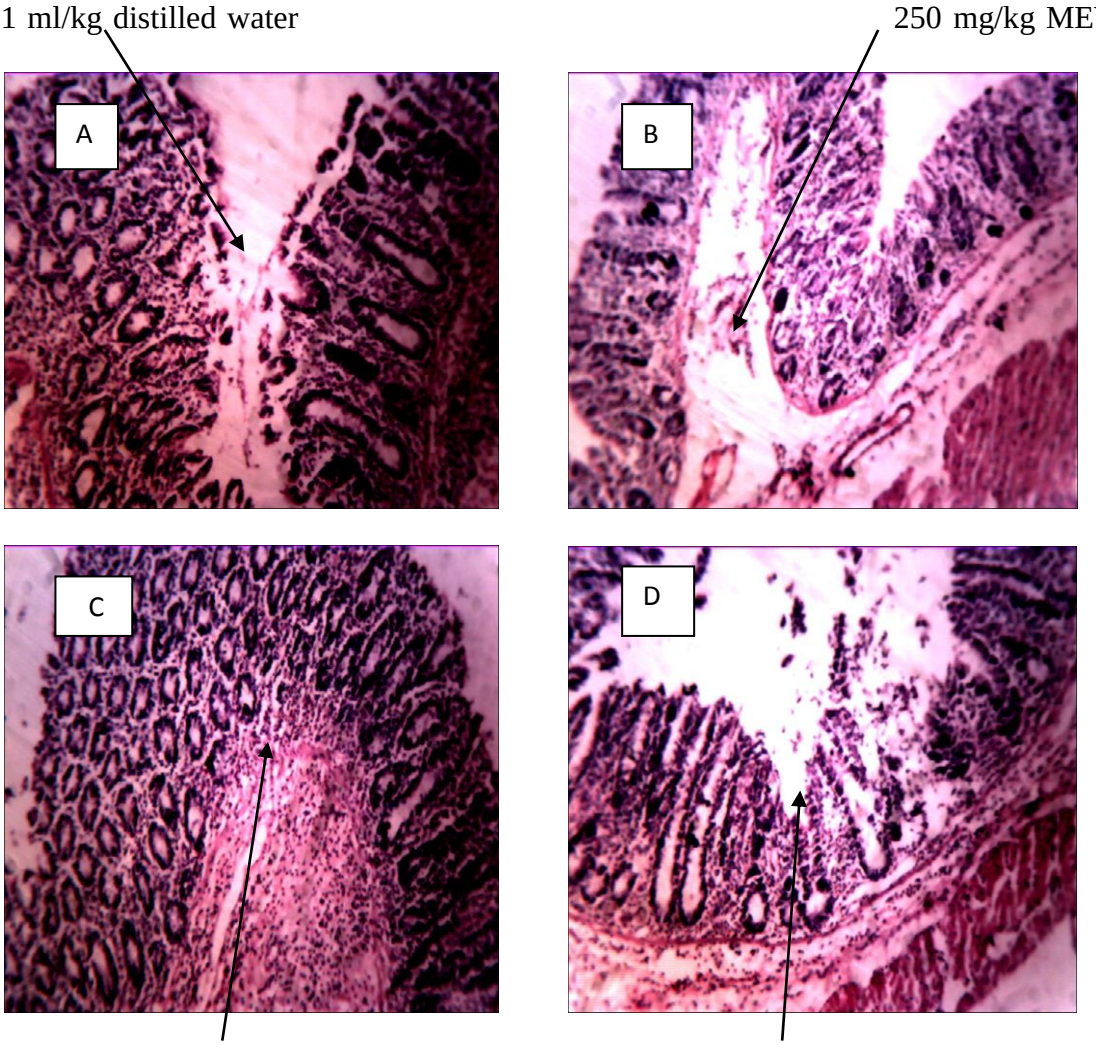


Plate IV A Photomicrograph of a section of the stomach of Wistar rat administered 1 ml/kg distilled water showing normal mucosa with glandular cells (H & E X 100)

Plate IV B Photomicrograph of a section of the stomach of Wistar rat administered 250 mg/kg methanol root extract of *Waltheria indica* showing normal mucosa and sub-mucosa with glandular cells (H & E X 100)

Plate IV C Photomicrograph of a section of the stomach of Wistar rat administered 500 mg/kg methanol root extract of *Waltheria indica* showing normal mucosa, sub-mucosa and muscularis with glandular cells (H & E X 100)

Plate IV D Photomicrograph of a section of the stomach of Wistar rat administered 1,000 mg/kg methanol root extract of *Waltheria indica* showing normal mucosa and sub-mucosa with glandular cells (H & E X 100)

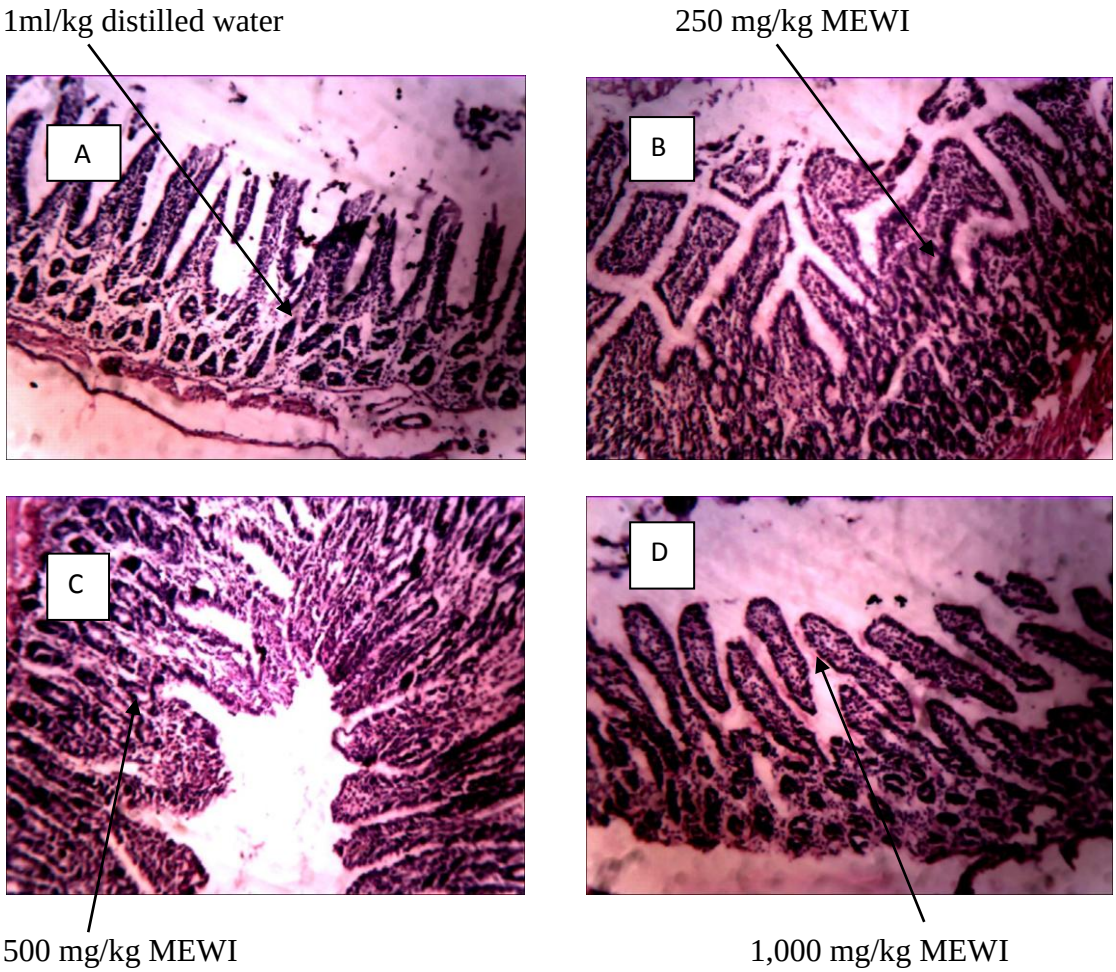


Plate V A Photomicrograph of a section of the intestine of Wistar rat administered 1 ml/kg distilled water showing normal intestinal architecture (H & E X 100)

Plate V B Photomicrograph of a section of the intestine of Wistar rat administered 250 mg/kg methanol root extract of *Waltheria indica* showing normal absorptive finger like projections (H & E X 100)

Plate V C Photomicrograph of a section of the intestine of Wistar rat administered 500 mg/kg methanol root extract of *Waltheria indica* showing normal absorptive glands and normal intestinal architecture with no obvious histology change (H & E X 100)

Plate V D Photomicrograph of a section of the intestine of Wistar rat administered 1,000 mg/kg methanol root extract of *Waltheria indica* showing normal absorptive glands and normal intestinal architecture with no obvious histology change (H & E X 100)

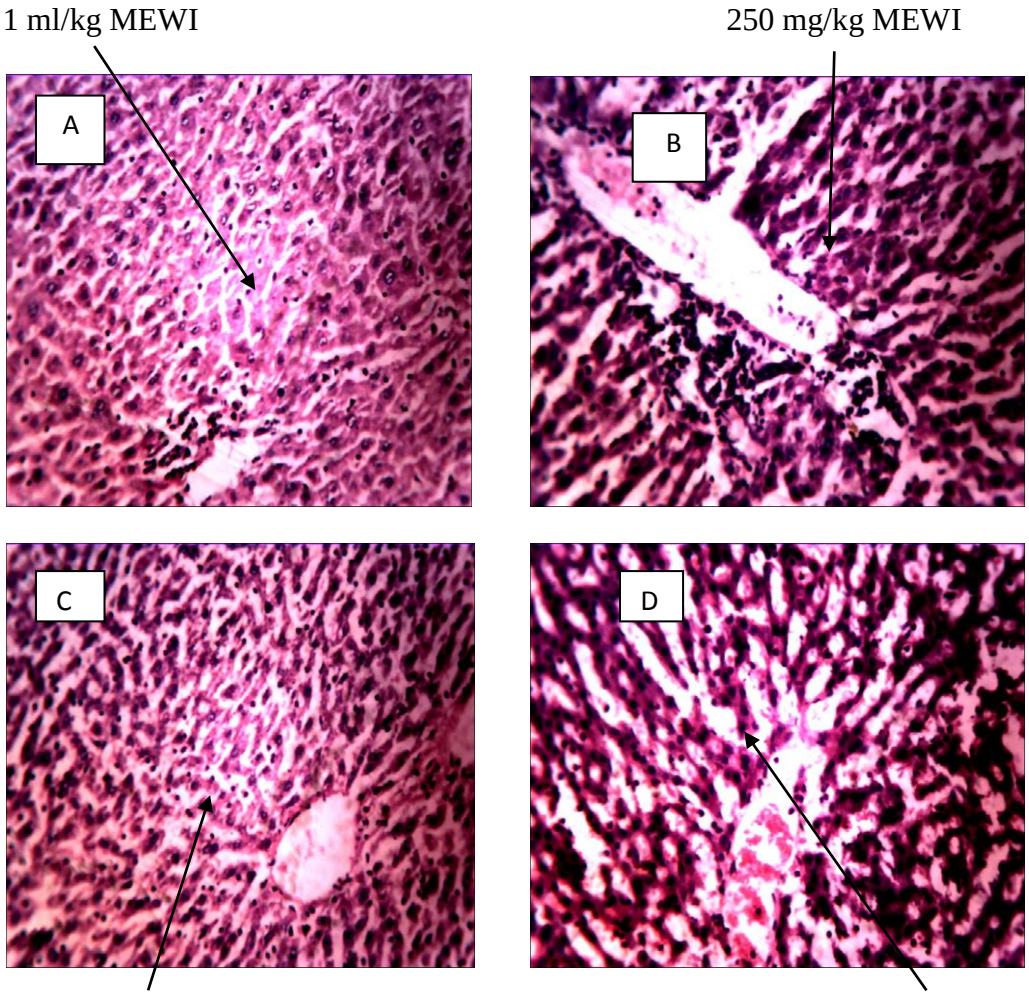


Plate VI A Photomicrograph of a section of the liver of Wistar rat administered 1 ml/kg distilled water showing normal hepatocytes (H & E X 250)

Plate VI B Photomicrograph of a section of the liver of Wistar rat administered 250 mg/kg methanol root extract of *Waltheria indica* showing polymorphonuclear infilteration (H & E X 250)

Plate VI C Photomicrograph of a section of the liver of Wistar rat administered 500 mg/kg methanol root extract of *Waltheria indica* showing moderate hepatocellular necrosis (H & E X 250)

Plate VI D Photomicrograph of a section of the liver of Wistar rat administered 1,000 mg/kg methanol root extract of *Waltheria indica* showing polymorphonuclear infiltration (H & E X 250)

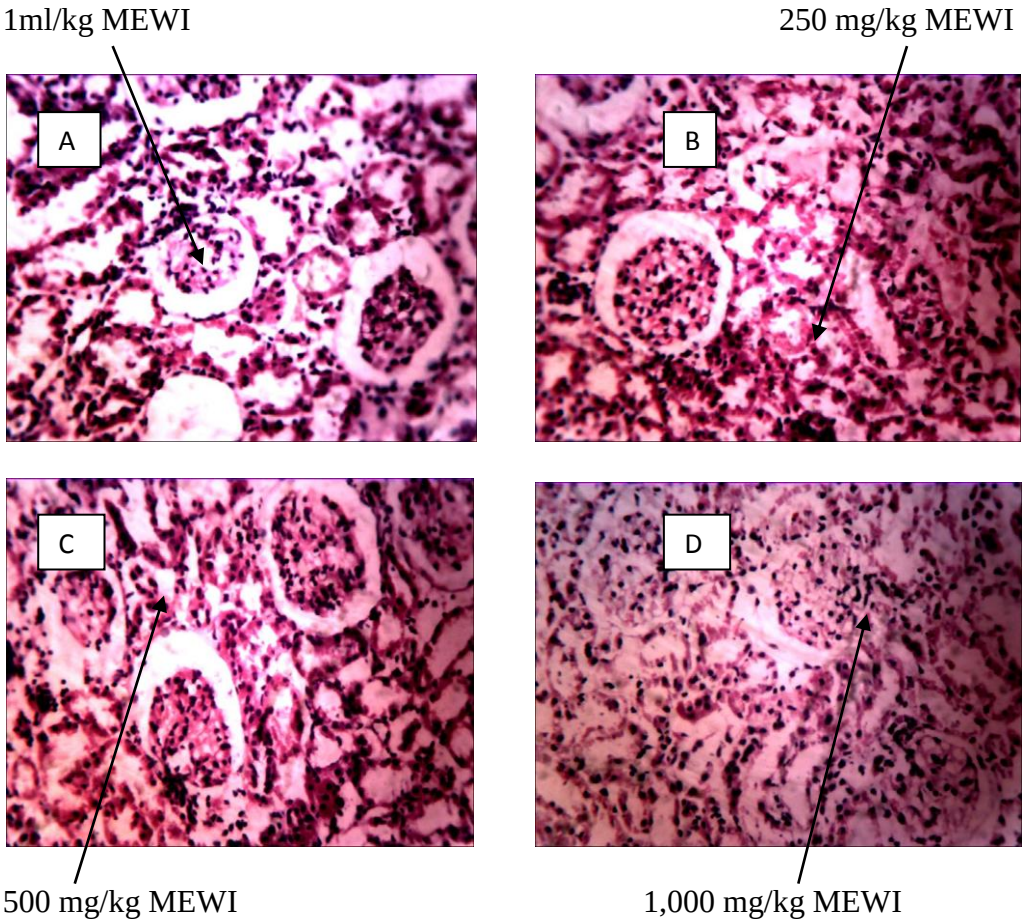


Plate VII A Photomicrograph of a section of the kidney of Wistar rat administered 1 ml/kg distilled water showing normal glomeruli and renal tubular structures (H & E X 250)

Plate VII B Photomicrograph of a section of the kidney of Wistar rat administered 250 mg/kg methanol root extract of *Waltheria indica* showing normal glomeruli and renal tubular structures (H & E X 250)

Plate VII C Photomicrograph of a section of the kidney of Wistar rat administered 500 mg/kg methanol root extract of *Waltheria indica* showing normal normal glomeruli and renal tubular structures (H & E X 250)

Plate VII D Photomicrograph of a section of the kidney of Wistar rat administered 1,000 mg/kg methanol root extract of *Waltheria indica* showing tubular necrosis (H & E X 250)

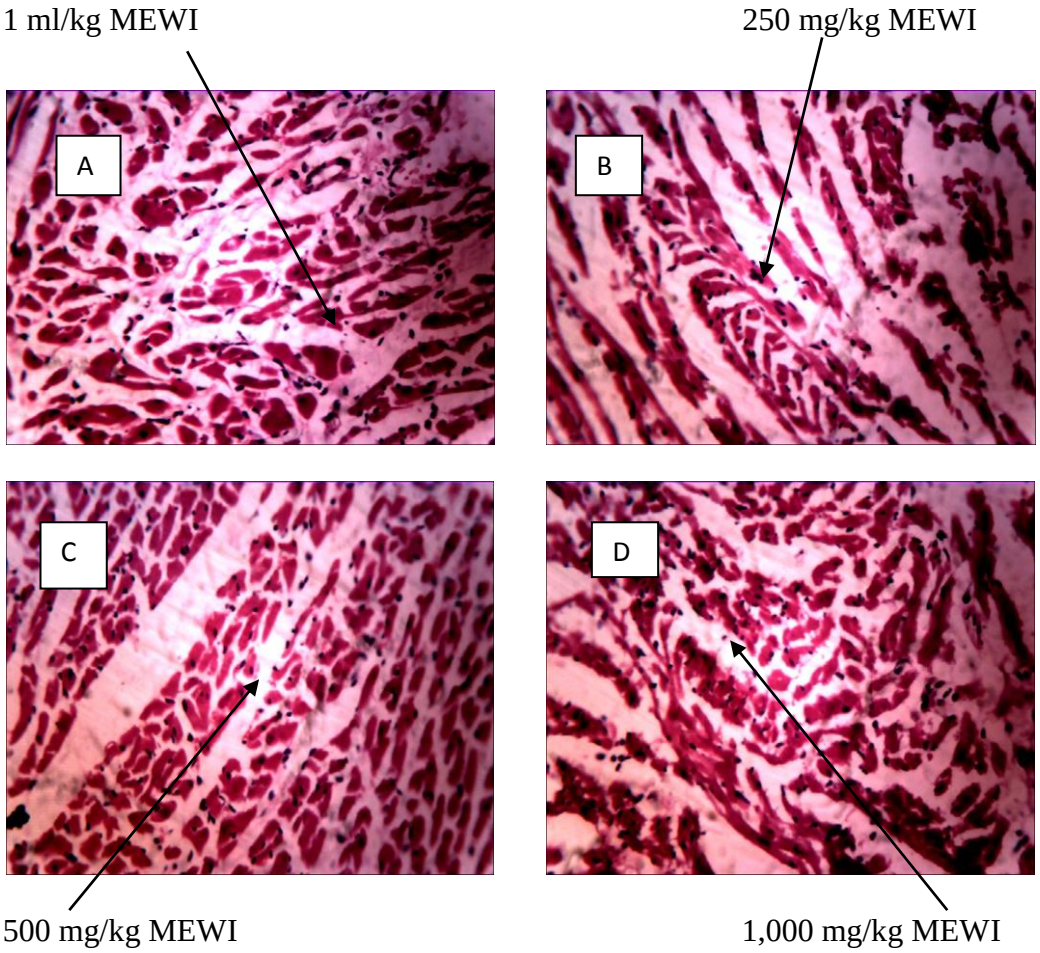


Plate VIIIA Photomicrograph of a section of the heart of Wistar rat administered 1 ml/kg distilled water showing no obvious histology change in the myocardium (H & E X 250)

Plate VIII B Photomicrograph of a section of the heart of Wistar rat administered 250 mg/kg methanol root extract of *Waltheria indica* showing no obvious histology change in the myocardium (H & E X 250)

Plate VIII C Photomicrograph of a section of the heart of Wistar rat administered 500 mg/kg methanol root extract of *Waltheria indica* showing no obvious histology change in the myocardium (H & E X 250)

Plate VIII D Photomicrograph of a section of the heart of Wistar rat administered 1,000 mg/kg methanol root extract of *Waltheria indica* showing no obvious histology change in the myocardium (H & E X 250)

4.6 Gastro-intestinal Mucosal Protective Studies in Wistar Rats

4.6.1 Indomethacin-induced ulcer in Wistar rats

The MEWI at 250 mg/kg reduced the number of ulcer sites and largest ulcer diameter but there was no statistical significant difference when compared with the negative control. There was significant ($p \leq 0.01$) reduction in the number of ulcer sites at doses of 500 and 1,000 mg/kg MEWI and 100 mg/kg cimetidine at $p \leq 0.001$. The largest ulcer diameter, was significantly reduced by 500 mg/kg MEWI at $p \leq 0.05$ and by 1,000 mg/kg and 100 mg/kg cimetidine at $p \leq 0.01$. The mean ± SEM sum of ulcer was significantly reduced by 250 mg/kg MEWI at $p \leq 0.05$ and by 500, 1,000 MEWI and 100 mg/kg cimetidine at $p \leq 0.001$ (Table 4.7).

4.6.2 Pylorus ligation induced-ulcer in Wistar rats

The MEWI at 250 mg/kg reduced the number of ulcer sites but there was no statistical significant difference when compared with the negative control. However, the extract at 500 mg/kg, 1,000 mg/kg and 20 mg/kg omeprazole significantly ($p \leq 0.05$; $p \leq 0.01$) decreased the number of ulcer sites. The gastric volume was significantly reduced at $p \leq 0.01$ by the 250 mg/kg extract group and at $p \leq 0.001$ by the 500, 1,000 MEWI and 20 mg/kg omeprazole group. Statistical significant decrease in ulcer diameter and ulcer index at all the dose points of the extract and omeprazole was seen at $p \leq 0.001$. There was statistical significant decrease in the volume of 0.1 N sodium hydroxide used and titratable acidity of 1,000 mg/kg MEWI at $p \leq 0.05$ and at $p \leq 0.001$ of the omeprazole group. Although there was a reduction in the volume of 0.1 N NaOH and titratable acidity at 250 mg/kg MEWI, it was not significantly different from control (Table 4.8).

Table 4.7: Effect of oral administration of methanol root extract of *Waltheria indica* on indomethacin-induced ulcer in Wistar rats

Treatment group	Number of ulcer sites	Largest ulcer diameter (mm)	Mean±SEM sum of ulcer (mm)	Percentage protection (%)
D.W. (1ml/kg)	10.83±2.12	2.0±0.52	11.5±2.30	0.00
MEWI (250 mg/kg)	6.00±1.39	1.25±0.36	5.40±1.50*	53.04
MEWI (500 mg/kg)	3.67±1.33**	0.75±0.31*	3.10±1.30***	73.04
MEWI (1,000 mg/kg)	3.00±1.09**	0.40±0.20**	2.00±0.90***	82.61
Cimetidine (100 mg/kg)	1.83±0.84**	0.30±0.20**	1.50±0.70***	86.96

Data is expressed as Mean ± SEM, n = 6,
*$p \leq 0.05$, **$p \leq 0.01$,*** $p \leq 0.001$ significant difference using one way ANOVA with Dunnett's post hoc test
D.W. = distilled water
MEWI = methanol root extract of *Waltheria indica*

Table 4.8 Effect of oral administration of methanol root extract of *Waltheria indica* on pylorus ligation-induced ulcer in Wistar rats

Treatment group	Number of ulcer sites	Gastric volume (ml)	Ulcer diameter (mm)	Ulcer index	Volume of 0.1N NaOH (ml)	Titratable acidity	Percentage protection (%)
D.W. (1 ml/kg)	4.75±1.12	6.98±0.51	6.25±1.11	17.00±1.08	0.78±0.09	77.50±4.54	0.00
MEWI (250 mg/kg)	1.75±1.18	3.90±0.50**	1.25±0.95***	5.00±3.00***	0.70±0.06	70.00±5.77	70.58
MEWI (500 mg/kg)	1.00±0.58*	1.40±0.49***	1.00±0.58***	4.00±2.31***	0.65±0.07	65.00±3.46	76.47
MEWI (1,000 mg/kg)	0.50±0.29**	1.33±0.19***	0.75±0.48***	3.50±2.02***	0.48±0.06*	47.50±3.29*	79.41
OMP (20 mg/kg)	0.25±0.25**	1.80±0.67***	0.50±0.50***	1.13±1.13***	0.33±0.06***	32.50±6.29***	93.35

Data is expressed as mean ± SEM, n=8

*$p \leq 0.05$, **$p \leq 0.01$, $p \leq 0.001$ (statistical significant difference using one way ANOVA with Dunnett's post hoc test

D.W. = distilled water

MEWI = methanol root extract of *Waltheria indica*

OMP = Omeprazole

4.7 Isolated Tissue Experiment

4.7.1 The effect of histamine on guinea pig ileum isolated preparation

The graded doses of histamine (10, 20, 40, 80 and 160 ng/ml) gave a log final organ bath concentration (FOBC) of 1, 1.3, 1.6, 1.9 and 2.2 respectively and dose dependent height of contraction as seen in figure 4.1a. The graded doses of histamine (100, 200, 400, 800 and 1,600 ng/ml) gave a log FOBC of 2, 2.3, 2.6, 2.9 and 3.2 respectively and also a dose dependent height of contraction as seen in figure 4.1b.

4.7.2 The effect of methanol root extract of *Waltheria indica* on guinea pig ileum isolated preparation

Graded doses of MEWI (20, 40, 80, 100, 200, 400, 800, 1,000, 2,000 µg/ml) did not contract/relax the guinea pig ileum.

4.7.3 The effect of acetylcholine on rabbit jejunum isolated preparation

The graded doses of acetylcholine (50, 100, 200, 400 and 800 ng/ml) gave a log FOBC of 1.7, 2.0, 2.3, 2.6 and 2.9 respectively and a dose dependent height of contraction.

4.7.4 The effect of methanol root extract of *Waltheria indica* on rabbit jejunum isolated preparation

The graded doses of MEWI (10, 20, 40, 80, 100, 200, 400, 800, 1,000, 2,000, 4,000 and 8,000 µg/ml) did not contract the rabbit jejunum.

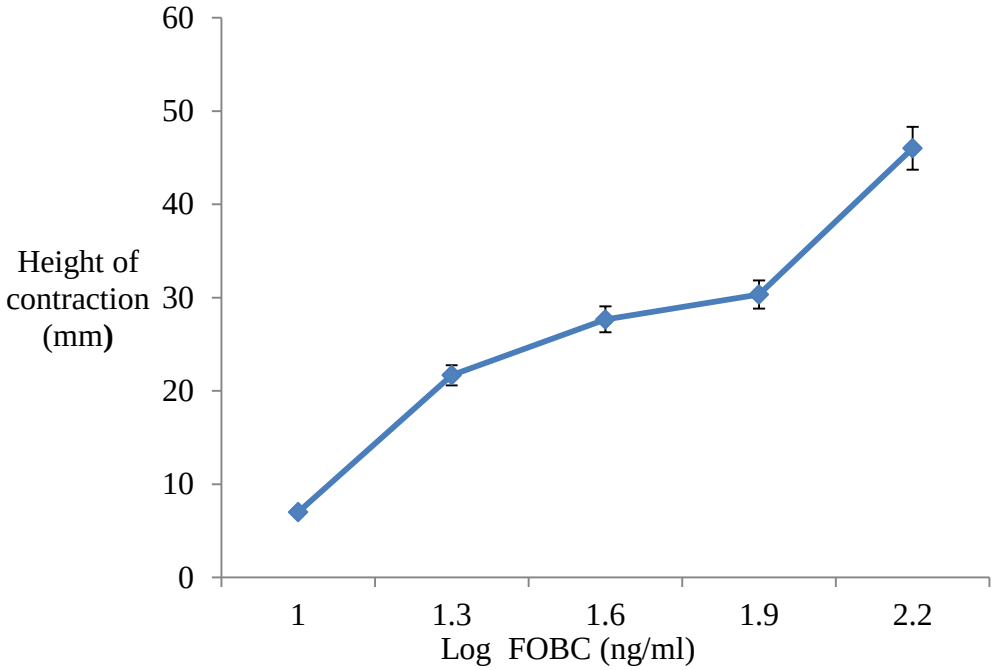


Fig 4.1a: Effect of histamine (10-160 ng/ml) final organ bath concentration on guinea pig ileum isolated preparation

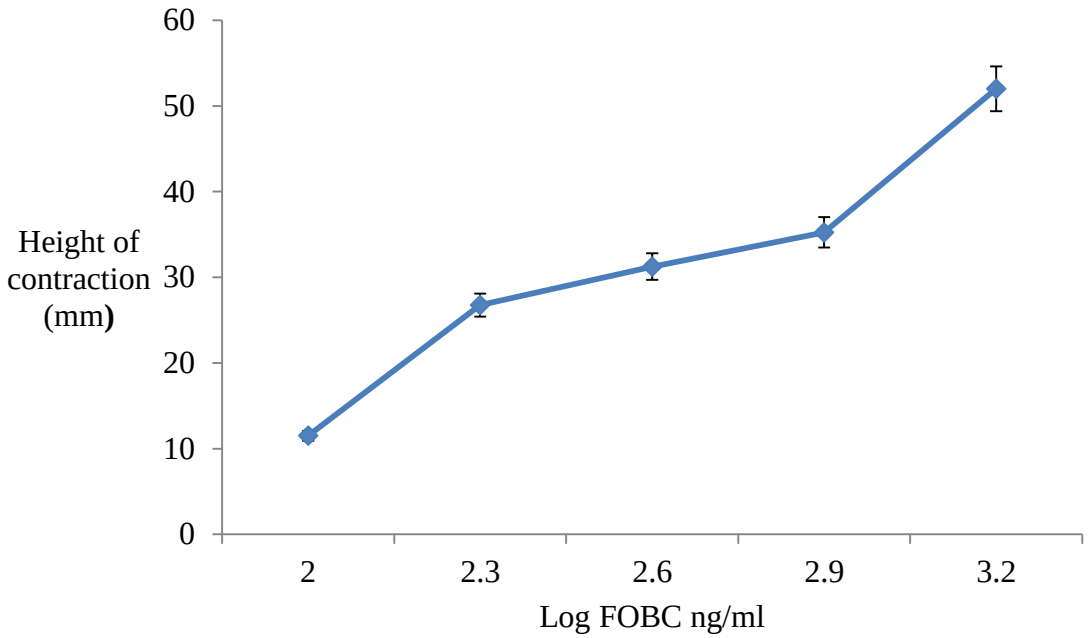


Fig 4.1b: Effect of histamine (100-1,600 ng/ml) final organ bath concentration on guinea pig ileum isolated preparation

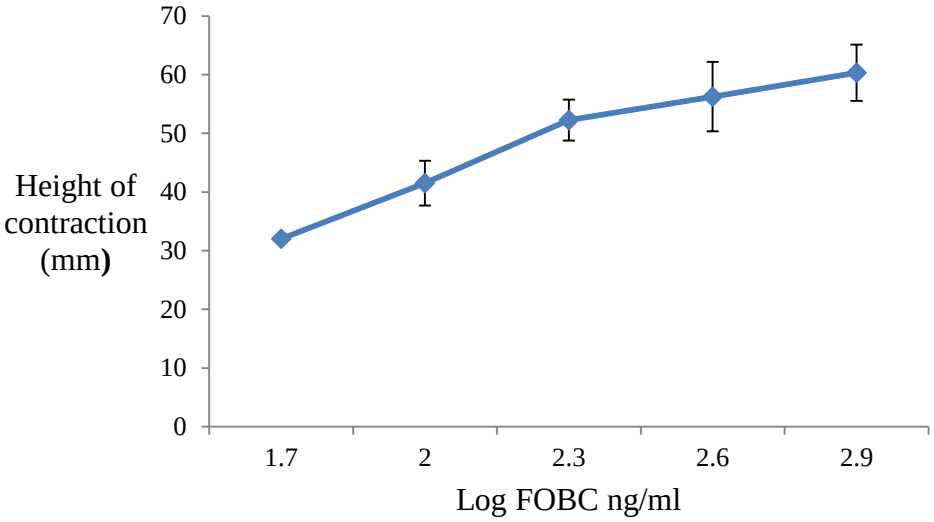


Fig 4.2: Effect of acetylcholine (50-800 ng/ml) final organ bath concentration on rabbit jejunum isolated preparation

CHAPTER FIVE

5.0 DISCUSSION

Preliminary phytochemical screening of the root extract showed the presence of secondary metabolites which are known to be responsible for the pharmacological activities of medicinal plants (Ojezele and Agunbiade, 2013). The therapeutic benefits of medicinal plants are usually attributed to the combination of different phytochemical constituents (Sasidharan *et al*., 2011). Alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponin glycoside, tannins and triterpenes were present in the MEWI while free anthracene and unsaturated steroids were absent. This finding is in line with that of Musa *et al* (2016) who reported the presence of these phytoconstituents in the plant.

The LD_{50} was estimated to be greater than 5,000 mg/kg orally indicating a high safety margin of the extract that is; the extract was practically non- toxic on oral administration. It is good to note that LD_{50} is an index of acute toxicity and should not be regarded as an absolute value as the values can change when certain conditions change. Some of such conditions are: species of the animals used, sex of the animals, environmental factors and repetition of the experiment.

Sub-acute toxicity study helps to evaluate the long term toxic effect of substances and it acts as a guide in selecting a safe dose for human use (Alli *et al.*, 2015). The 28-day sub-acute toxicity study showed no significant difference in the weight of the treated rats when compared with control indicating that the basic metabolic processes of the rats were not adversely affected by the daily administration of the extract. Changes in body weights are good indicators of adverse effects of drugs. The ROW ratio of the stomach, intestine, liver, kidney and heart were not affected by the daily administration of MEWI indicating that

W. indica was not toxic to these organs. Changes in organ weights are good indicators of treatment related effects of test substances (Sellers *et al*., 2007).

Analysis of blood parameters is important in the evaluation of risks associated with test compounds under investigation and it gives an idea of the physiological and pathological status of man and animals (Jothy *et al*., 2011). The haematological parameters investigated (PCV, Hb, RBC, platelets, WBC, neutrophils, lymphocytes, monocytes and eosinophil differential count) at all the extract doses were not affected by the daily administration of the extract indicating that the extract was not toxic to blood cells (Amala Hazel *et al.,* 2016).

ALT and AST are good indicators of liver function. They are largely used in the assessment of liver damage by drugs or hepatotoxic substances. Destruction of liver cells, leads to the elevation of serum concentrations of these enzymes (Giannini *et al*., 2005). While AST is seen in the liver and other organs like skeletal muscle, heart, kidneys, brain, pancreas and blood cells, ALT is distinctively abundant in the cytoplasm of the liver cells therefore more commonly used as a marker for suspected liver cell damage (Bello *et al*., 2016). There was an increase in ALT at 250 mg/kg MEWI indicative of possible liver damage caused by the daily oral administration of the extract. The increase in ALT at 250 mg/kg MEWI is a dose specific effect.

Bilirubin, is the break down product of haemoglobin. The water-insoluble unconjugated bilirubin formed is then transported to the liver and conjugated and then excreted into the bile (Giannini *et al*., 2005). Increased bilirubin level is used to diagnose liver disease and blockage of bile duct. There was an increase in T.Bil at all the dose points of MEWI and an

increase in conjugated bilirubin at 1,000 mg/kg MEWI. This increase may be due to injury or destruction of liver hepatocytes by the extract.

Serum electrolytes, urea and creatinine are markers of renal excretory function (Alli *et al*., 2015). The daily administration of the extract had no significant effect on the electrolytes investigated (Na^+, K^+, Cl^-, and $HCO3^-$) implying that the extract had no adverse effect on renal function at the doses tested.

Urea and creatinine are waste products of protein and muscle metabolism respectively and both are excreted by the kidney (Schwart and Garrison, 2008). The kidneys regulate the reabsorbtion of electrolytes into the blood and excretion of urea and creatinine. Filtration and absorbtion takes place in the glomeruli and renal tubules respectively so when there is compromise of normal glomeruli function, substances normally cleared by the kidneys such as urea and creatinine, accumulate in the blood. Therefore, the absence of any significant change in urea and creatinine in this study, suggests that the normal excretion of these waste products by the kidney was not affected by the different dose points of MEWI indicating normal kidney excretory function.

Histology studies are usually carried out to provide supportive evidence on the results obtained from haematological and biochemical analysis (Kumar *et al*., 2014). In the histology examination, no obvious morphological changes were seen in the stomach, intestine and heart of Wistar rats at all dose points of MEWI. However, the liver showed polymorphonuclear infiltration at 250 and 1,000 mg/kg MEWI and moderate hepatocellular necrosis at 500 mg/kg. The kidney showed no obvious histological change at 250 and 500 mg/kg but tubular necrosis was seen at 1,000 mg/kg MEWI. The liver and kidneys are

target organs for toxic substances due to their bodily functions of metabolism/detoxification and excretion respectively. They have high sensitivity to toxic substances hence are able to predict toxicity (Bello *et al*., 2016). The changes observed in the histology examination of the liver and kidney of Wistar rats were moderate. They indicated that the extract might have slight toxic potential on the liver and kidney at increasing doses. It could be that the histology lesions caused by the extract on the liver and kidney, were not serious enough to have been seen in most of the measured parameters. Therefore, it is most likely that the chemical constituents of the MEWI might have interacted directly with the cells of these organs to produce the histology change which was not seen in the blood and renal function indices.

Indomethacin is a known ulcerogen of the gastric mucosa and it is considered to be the most common NSAID known to induce experimental gastric ulcer (Suleyman *et al*., 2010). Indomethacin induced-ulcer model is the most widely used model due to the fact that NSAIDs are the second most common cause of PUD. They cause ulcers by inhibiting prostaglandin synthetase via cyclooxygenase pathway thus compromising the function of prostaglandins which include: stimulation of bicarbonate and mucous secretion, maintaining mucosal blood flow and regulating mucosal cell turn over and repair. This ulcer model is used to investigate the potential usefulness of anti-secretory and cytoprotective agents. The extract, was observed to significantly reduce the number of ulcer sites, ulcer diameter and mean sum of ulcer dose dependently. It also gave a percentage protection of 53.04, 73.04 and 82.81% at 250, 500 and 1,000 mg/kg respectively. This showed that the higher the dose of MEWI, the greater its ability to protect the stomach from

ulcer caused by indomethacin. Futhermore, the protection conferred by the 1,000 mg/kg MEWI group was comparable to that of 100 mg/kg cimetidine which was 86.96%.

Pylorus ligation is one of the most widely used methods for studying the effect of drugs on gastric secretion. Ligation of the pyloric end of the stomach causes accumulation of gastric acid which leads to auto digestion of the gastric mucosa and breakdown of the mucosal barrier and finally the development of ulcers. This model is also useful for evaluating the effects of antisecretory drugs. The number of ulcer sites, gastric volume, ulcer diameter, volume of 0.1 N NaOH, ulcer index and titratable acidity were reduced by the MEWI. A percentage protection of 70.85, 76.47 and 79.41% obtained from 250, 500 and 1,000 mg/kg MEWI respectively showed that the MEWI at all dose points gave good and dose dependent protection against pylorus ligation-induced ulcer indicating that the extract was more effective at higher doses.

The indomethacin and pylorus ligation-induced ulcer studies showed that the higher the dose of the extract, the greater the gastro-intestinal mucosal protection but there may be concerns of possible dose-related toxic effects on the liver and kidney.

Alkaloids, flavonoids, saponins, tannins and triterpenes have been documented to possess anti-ulcer activity and these bioactive constituents were present in the MEWI. Alkaloids decrease the gastric content and gastric acidity by increasing mucosal prostaglandins which in turn increases mucous and bicarbonate secretion (Falcao *et al*., 2008). Flavonoids are cytoprotective reported to act via several mechanisms such as increased mucosal cell turnover, increased gastric blood flow, bactericidal activity against *H. pylori* and anti-secretory action to protect the gastric mucosa (Di carlo *et al*., 1999). Saponins form a

protective mucous layer on the gastric mucosa thereby preventing erosion by gastric acid (Amazu *et al*., 2015). Tannins are astringent in nature which means that they prevent ulcer development by precipitating proteins on the ulcer site that is they form complexes with water insoluble proteins thus protecting the mucosa from gastric acid (Sumbul *et al*., 2011). The presence of these bioactive constituents in the root of *Waltheria indica* is probably responsible for its gastro-intestinal mucosal protective effect against indomethacin and pylorus ligation-induced ulcers.

Isolated tissue experiments are in-vitro experiments to mimic what happens inside the body for a better understanding of the pharmacokinetics of drugs and chemicals. On the guinea pig ileum, histamine gave a concentration dependent contraction at 20-1,600 ng/ml FOBC. Acetylcholine also gave a concentration dependent contraction at 100-800 ng/ml FOBC on the rabbit jejunum. The MEWI did not contract/relax the guinea pig ileum. Akah *et al* (2007) and Ezike *et al* (2014) reported that *Ocimmum graticimmum* and *Desmodium velutinum* extracts respectively neither contracted nor relaxed the isolated guinea pig ileum however, the extracts inhibited the contractions evoked by histamine. Also, the MEWI gave no contraction/relaxation at 10-8,000 µg/ml FOBC on the rabbit jejunum. The consistent no contraction or relaxation obtained may be due to the antagonistic effect of the MEWI on histaminic and muscarinic receptors on the isolated guinea pig ileum and rabbit jejunum respectively although the interaction studies were inconclusive due to power failure, out dated equipment and inconsistency of result.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The findings of this study suggest that the MEWI contains bioactive compounds that have significant gastro-intestinal mucosal protective properties. The protective properties could be through anti-secretory and mucosal protection mechanisms as seen in indomethacin-induced ulcer, pylorus ligation induced-ulcer and the isolated tissue experiment. All these, may account for the use of *W. indica* in herbal medicine in the prevention and treatment of PUD.

6.2 Recommendations

i. Fractionation of the bioactive constituents responsible for the gastro-intestinal mucosal protective activity of the plant.

ii. Elucidation of the chemical structure of the bioactive constituents.

iii. There may be need for long-term toxicity studies on the root extract of the plant.

iv. Further pharmacological investigations are necessary to determine other possible mechanisms of action of the plant using other models like *Helicobacter pylori*-induced ulcer and ethanol-induced ulcer models among others.

v. Interaction studies with histamine, mepyramine and different concentrations of MEWI on the guinea pig ileum as well as acetylcholine, atropine and different concentrations of MEWI on the rabbit jejunum should be carried out to further clarify the mechanism of action of the MEWI.

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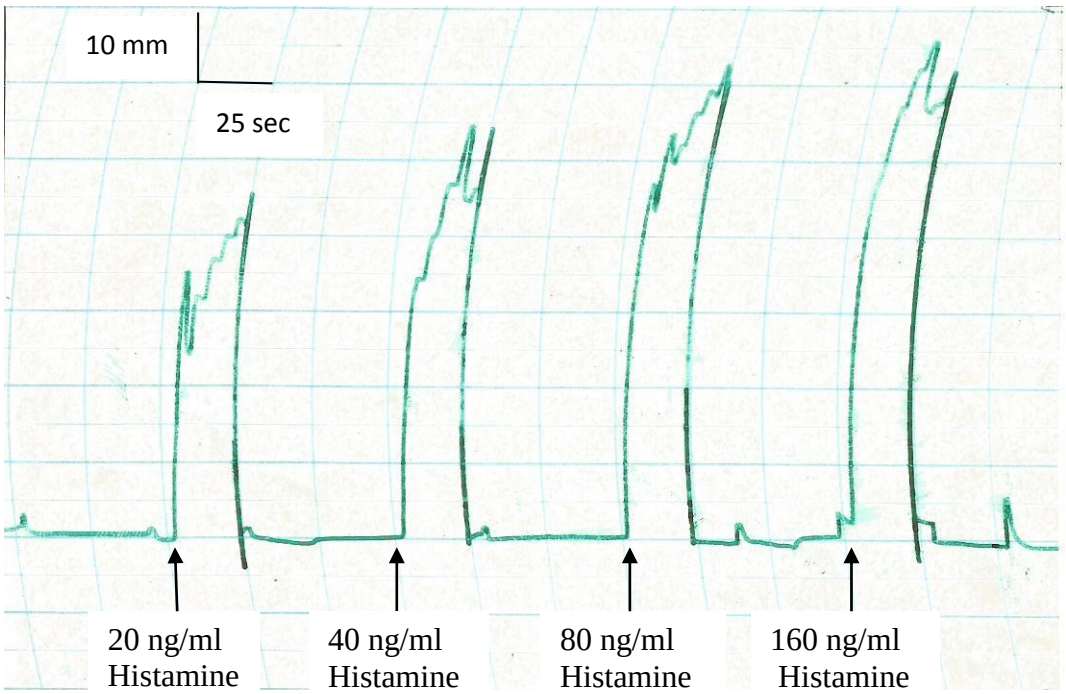
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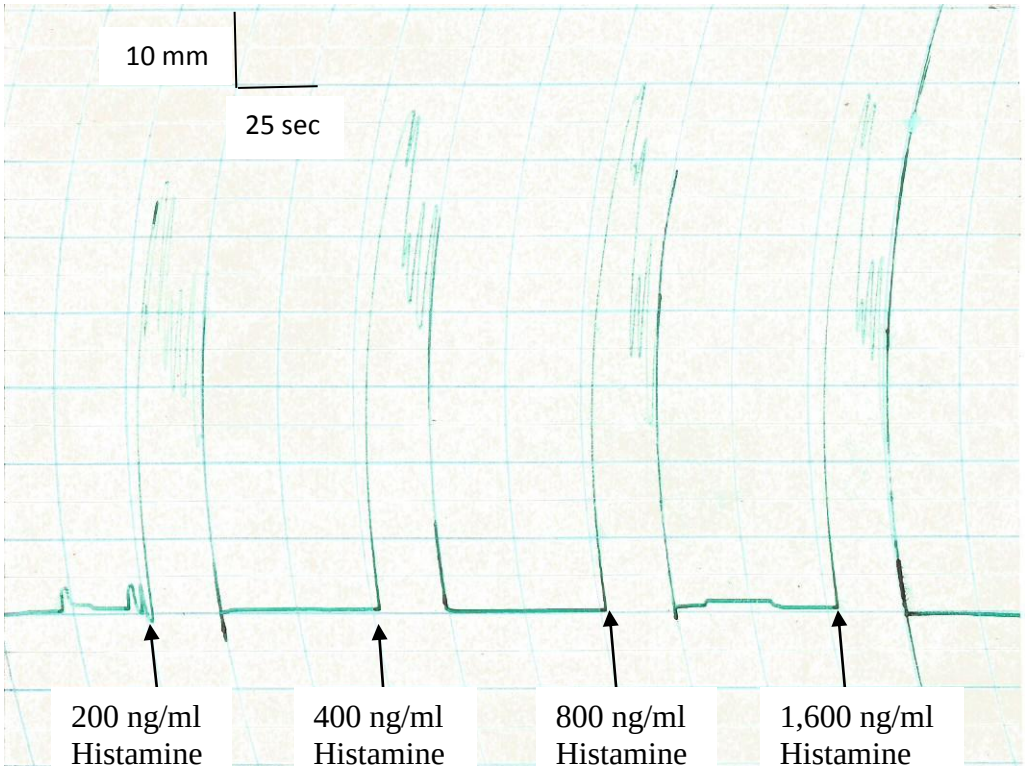
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APPENDICES

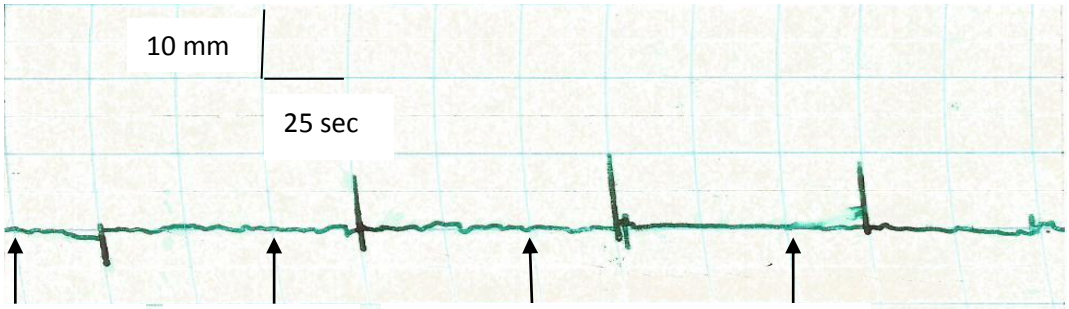
Appendix 1: Effect of histamine (20-160 ng/ml) final organ bath concentration on isolated guinea pig ileum



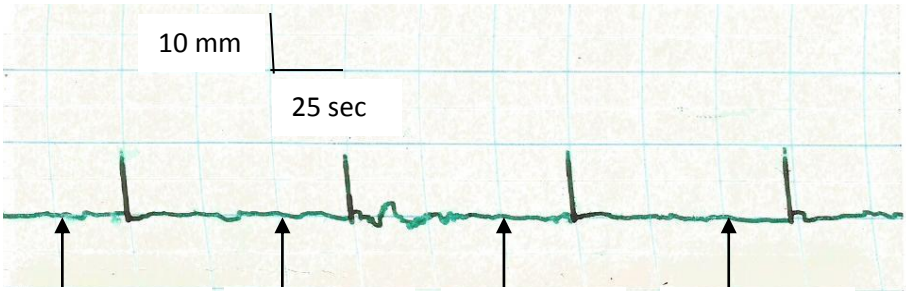
Appendix 2: Effect of histamine (200-1,600 ng/ml) final organ bath concentration on isolated guinea pig ileum



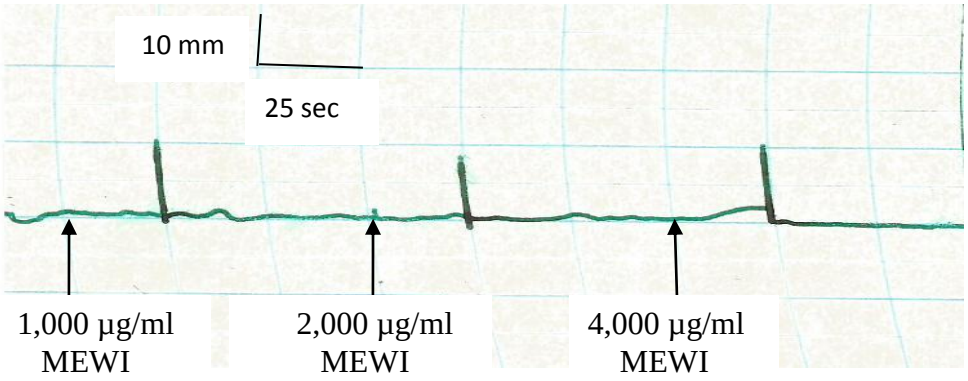
Appendix 3: Effect of methanol root extract of *Waltheria indica* (10-80 µg/ml) final organ bath concentration on isolated guinea pig ileum



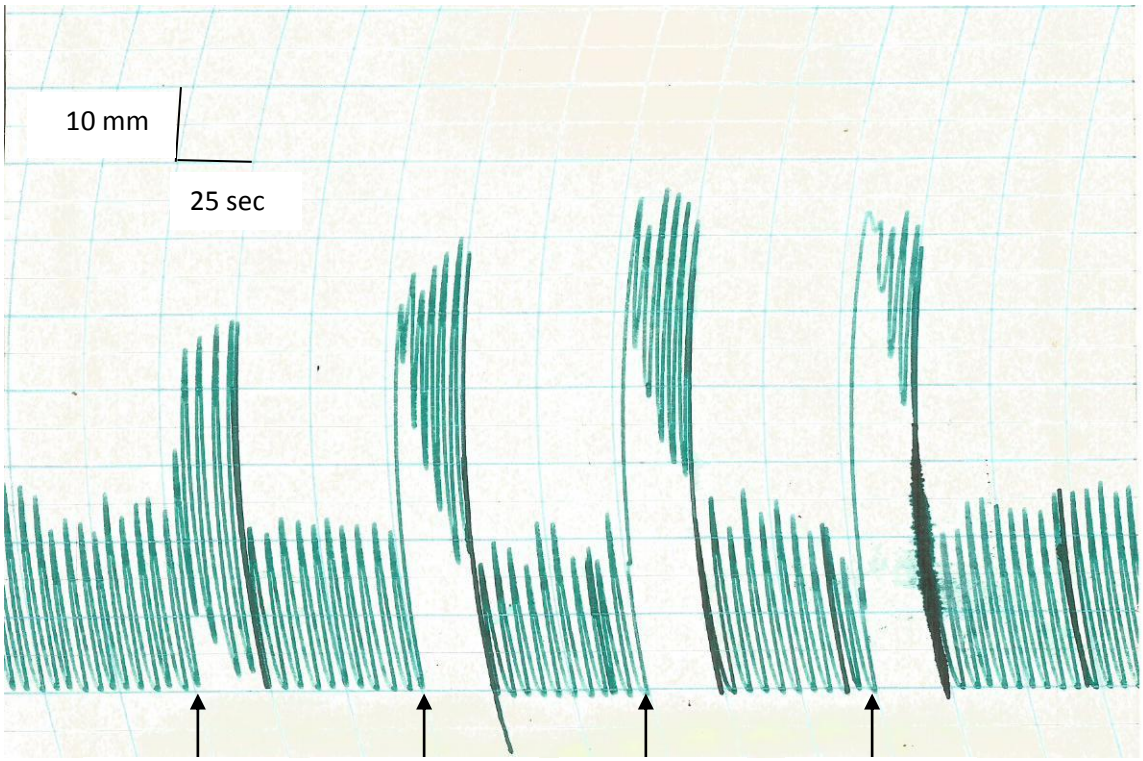
Appendix 4: Effect of methanol root extract of *Waltheria indica* (100-800 µg/ml) final organ bath concentration on isolated guinea pig ileum



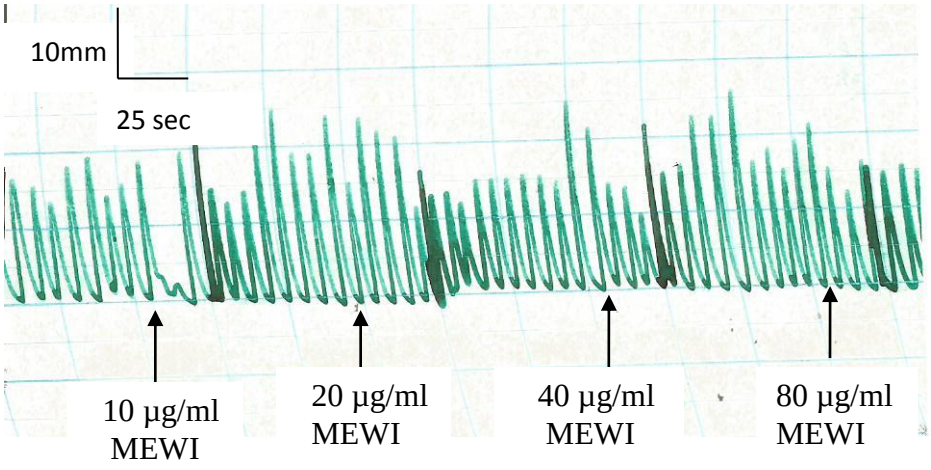
Appendix 5: Effect of methanol root extract of *Waltheria indica* (1,000-4,000 µg/ml) final organ bath concentration on isolated guinea pig ileum



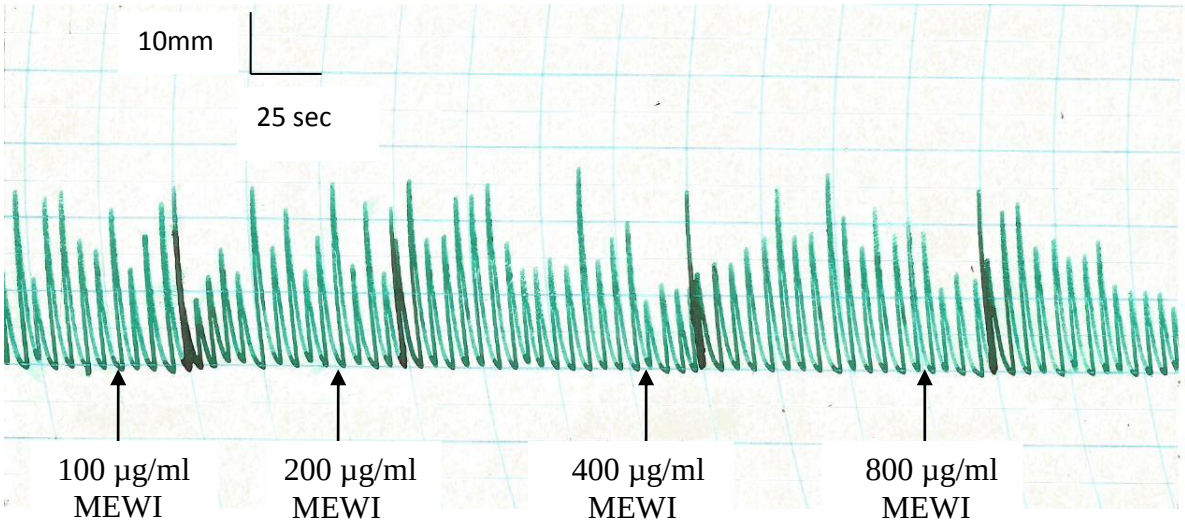
Appendix 6: Effect of acetylcholine (100-800 ng/ml) final organ bath concentration on isolated rabbit jejunum



Appendix 7: Effect of methanol root extract of *Waltheria indica* (10-80 µg/ml) final organ bath concentration on isolated rabbit jejunum



Appendix 8: Effect of methanol root extract of *Waltheria indica* (100-800 µg/ml) final organ bath concentration on isolated rabbit jejunum



Appendix 9: Effect of methanol root extract of *Waltheria indica* (1,000-8,000 µg/ml) final organ bath concentration on isolated rabbit jejunum

