Department of Pharmacology and Clinical Pharmacy

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A thesis paper submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University in partial fulfillment of the requirements for the Master of Science degree in pharmacology

Under the supervision of: Teshome Nedi (PhD, Associate professor)

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Ousman Ahmed, entitled "Evaluation of anti-gastric ulcer activity of aqueous and 80% methanol leaf extracts of *Urtica simensis* in rats" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Evaluation of anti-gastric ulcer activity of aqueous and 80% methanol leaf extracts of *Urtica simensis* in rats.

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Addis Ababa University, 2019

In Ethiopian traditional medicine, the leaf of *Urtica simensis* is used in the treatment of gastric ulcer. Since this claim has not been investigated scientifically, this study was undertaken to evaluate the anti-gastric ulcer activity of both aqueous and 80% methanol extracts of *Urtica simensis*. The aqueous and 80% methanol leaf extracts of *Urtica simensis* were prepared using decoction and maceration techniques of extraction respectively. Three models, pylorus ligation, indomethacin and ethanol induced gastric ulcer models were employed to assess the anti-secretory and mucoprotective potential of the extracts. Three dose levels (100, 200, and 400 mg/kg) were employed for each extracts. Negative control received distilled water, whereas positive controls were given cimetidine 100 mg/kg. In pylorus ligation induced gastric ulcer model, both types of extracts at dose of 200 &400 mg/kg were exhibited significant reduction in total acidity, volume of gastric secretion ($p<0.001$) and significant rise in pH ($p<0.05$) of the gastric secretion. In indomethacin induced ulcer model, both aqueous and methanol extracts were exhibited dose dependent increment in gastric wall mucus as compared to ulcerated control ($p<0.001$). In ethanol induced ulcer model, all doses of extract produced significant increment in gastric wall mucus from 46.66 ± 0.96 (aqueous100) to 75.87 ± 1.52 (methanol400) µg alcian blue/g wet stomach. Five days pre-treatment study with aqueous200 and methanol200 exhibited significant ($P<0.001$) ulcer inhibition in both indometacine and ethanol-induced ulcer model. This study revealed that both aqueous and 80% methanol leaf extracts of *Urtica simensis* is endowed with a promising anti-gastric ulcer activity in all of the above mentioned three models and these findings provide a scientific support for folkloric use of *Urtica simensis* leaf as treatment of gastric ulcer.

Key terms: Gastric ulcer disease, Indomethacin induced gastric ulcer, Pylorus ligation induced gastric ulcer, Ethanol induced ulcer model, *Urtica simensis*

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ACRONYM AND ABBREVIATIONS

AAU	Addis Ababa university
Ach	Acetylcholine
ADP	Adenine diphosphate
AMP	Adenine monophosphate
ANP	Atrial natriuretic peptide
AQ	Aqueous extract
ATP	Adenine triphosphate
cAMP	3´-5´-cyclic adenosine monophosphate
CCK	Cholecysokinin
CCK2R	Cholecystokinin receptor sub type 2
CGRP	Calcitonin gene-related peptide
CI	Cimetidine
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CRH	Corticotropin-releasing hormone
EC	Enterochromaffin
ECL	Enterochromaffin-like
EGFR	Epidermal growth factor receptor
GERD	Gastro esophageal reflux disease
H+/K+-ATPase	H+/K+-adenosine triphosphatase
H2R	Histamine 2 receptor
HCl	Hydrochloric acid
HSP	Heat shock proteins

H. pylori	Helicobacter pylori
LB4	Leukotriene B4
PGE2	Prostaglandin E2
PGI2	Prostacyclin
M3R	Muscarinic-3-receptor
ME	80% methanol extract
NO	Nitric oxide
NSAID	Non-steroidal anti-inflammatory drugs
PPIs	Proton pump inhibitors
PG	Prostaglandin
PUD	Peptic ulcer disease
ROS	Reactive oxidative state
SRMD	Stress-related mucosal damage
TRH	Thyrotrophic- releasing hormone
U. simensis	Urtica simensis
UI	Ulcer index
UN	Average number of ulcer per animal
UP	Percentage of animals with ulcer.
US	Average of severity score
VIP	Vasoactive intestinal peptide

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1. INTRODUCTION

1.1. Overview of peptic ulcer disease

Ulcers are lesions of the skin or mucous membrane characterized by the superficial inflamed dead tissue. Among different types of ulcers that can develop; peptic ulcers are the most common, it is the areas of degeneration and necrosis of gastrointestinal mucosa exposed to hydrochloric acid and peptic secretions though they can occur at any level of the alimentary tract. There are two types of PUDs based on their anatomical origin as gastric or duodenal. They occur most commonly (98-99%) in either the small intestine (duodenal ulcer) or the stomach (gastric ulcer) in the ratio of four to one (Mohan, 2010).

Gastric ulcer, also called stomach ulcer, is a break in the normal gastric mucosa integrity that extends through the muscularis mucosa into the sub mucosa or deeper and it is common in older age group. Symptoms may include nausea, vomiting, and weight loss. Although patients with gastric ulcers have normal or diminished acid production, yet ulcers may occur even in complete absence of acid and eating may increase pain rather than relieve pain in gastric ulcer (Vimala & Gricilda Shoba, 2014). Duodenal ulcer, an ulcer is present in the duodenum or the first part of intestine and the pain is often described as a burning or dull ache, belching, vomiting, weight loss, or poor appetite and the symptoms improve with eating. About a third of older people have no symptoms. Complications may include bleeding, perforation, and obstruction of the stomach (Roy, 2014).

Acid-related diseases (gastritis, erosions, and peptic ulcer) of the upper GI tract require gastric acid for their formation. Peptic ulcer disease (PUD) differs from gastritis and erosions in that ulcers typically extend deeper into the muscularis mucosa. Risk factors are psychological stress, physiological stress, H. pylori, drug intake (e.g. aspirin, steroids, butazolidine, indomethacin) and local irritants (e.g. alcohol, smoking, coffee etc (Mohan, 2010).

There are three common forms of peptic ulcers such as Helicobacter pylori positive, Non-steroidal anti-inflammatory drug (NSAID) induced, and stress-related mucosal damage (SRMD).H. pylori–

positive and NSAID-induced ulcers are chronic peptic ulcers that differ in etiology, clinical presentation, and tendency to recur. These ulcers develop most often in the stomach and duodenum of ambulatory patients. Occasionally, ulcers develop in the esophagus, jejunum, ileum, or colon. The natural course of chronic PUD is characterized by frequent ulcer (Adinortey, Ansah, & Galyuon, 2013; Dipiro JT et al., 2014).

1.2. Gastric acid secretion and its regulation

The gastric epithelium is a complex structure that consists of tubular branched gastric glands with a variety of cell types and neurons. It consists of three distinct anatomical parts, fundus, corpus and antrum and two functional parts, such as pyloric (gastrin cells) and oxyntic (parietal cells) glands. The oxyntic gland comprises 80% of the stomach and it contains the anatomy of fundus and corpus, pyloric gland area comprises the remaining 20% of the stomach and it contains the anatomy of antrum. Parietal cells secrete hydrochloric acid at a concentration of about 160 mM or about pH 0.8 and median daily pH in the human stomach is about 1.4 (Schubert & Peura, 2008).

The stomach performs a variety of functions including serving as a reservoir for food, exposing ingested food to acid and pepsin and providing a barrier that prevents microorganisms from entering the intestines. In addition, the stomach is exceedingly rich in the following peptide hormone-producing cells containing hormonal and paracrine signaling agents that regulate the activity of the parietal cell reside within the glands. These include D cells (somatostatin), Enterochromaffin-like (ECL) cells (histamine), A-like cells, Enterochromaffin (EC) cells (serotonin) and G cells (gastrin)(Mitchell & Schubert, 2008).

Parietal cell acid secretion is initiated by a variety of factors related to food ingestion and its regulation is via central, peripheral and cellular mechanisms. Acid is produced by the carbonic anhydrase-mediated catalysis of carbon dioxide (CO_2) and water (H_2O) to form hydrogen ion (H^+) and bicarbonate(HCO_3^-) . H^+ ions are then exchanged for potassium ions (K^+ by the H^+/K^+-adenosine tri-phosphatase (H^+K^+ ATPase) pump and later coupled with chloride ions (Cl^-) entering the parietal cell from the blood in exchange for HCO3-(Mejia & Kraft, 2009).

The rate of formation and secretion of hydrochloric acid by the parietal cells is directly related to the amount of histamine secreted by the ECL cells. In turn, the ECL cells can be stimulated to secrete histamine in several different ways: probably the most potent mechanism for stimulating histamine secretion is by the hormonal substance gastrin secreted by gastrin cell (G cells),which is formed almost entirely in the antral portion of the stomach mucosa in response to proteins in the foods being digested(Guyton & Hall, 2006). It stimulates histamine secretion from the ECL via cholecysokinin-2 (CCK2) receptors on the ECL surface membrane. Histamine then activates the histamine 2 receptor (H2R) on the parietal cells, resulting in gastric acid secretion (Bitziou & Patel, 2012).

The vagus nerve contains 80%–90% afferent fibers and 10%–20% efferent fibers that innervate the stomach. The efferent fibers are preganglionic and do not directly innervate parietal or neuro endocrine cells but rather synapse with postganglionic neurons in the wall of the stomach (Schubert & Peura, 2008).These neurons contain neurotransmitters, such as acetylcholine (Ach), gastrin-releasing peptide (GRP), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP), nitric oxide (NO) and substance P. Through these messengers, postganglionic neurons regulate acid secretion directly by influencing the parietal cell, or indirectly by modulating the secretion of hormonal and paracrine ligands (Smith, Dhatt, & Buchan, 2001).

Ach is released from postganglionic neurons of the enteric nervous system (ENS) and directly stimulates acid secretion by binding to muscarinic (M3) receptors on parietal cells. It may also stimulate acid secretion indirectly by inhibiting the release of somatostatin through activation of M2 and M4 receptors on D cells (Schubert & Peura, 2008). Somatostatin is authoritative physiological inhibitor of gastric acid secretion. It is synthesized and released from cytoplasmic processes of the D cells of the fundic and antral mucosa. These processes are in close contact with their target cells (e.g. gastrin, ECL, and parietal cells) and although secreted via the local circulation and ultimately acts on its target. A-like cells release ghrelin that stimulate acid secretion, through direct stimulation of parietal cell to release histamine and indirectly by inhibiting the activation of somatostatin receptors (Feldreich, 2010).

Neuro-hormonal secretagogues such as Histamine, Gastrin and Ach regulate acid secretion under the direct involvement of ECL cells and each of these secretagogues binds to and activates specific receptors on the basolateral membrane of the parietal cell, thereby initiating the biochemical events which are necessary for active transport of H+ out of the cell (Vijay Kumar Bansal et al., 2009).

1.3. Pathogenesis of peptic ulcer disease

Peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors (acid, pepsin, H. pylori and non-steroidal anti-inflammatory agents) and the mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins)(Zatorski, 2017).

1.3.1. Endogenous defense mechanism

Mucosal defence has been best characterized in the stomach, which exhibits remarkable resistance to the damaging effects of acid and pepsin. The first level of defence consists of the factors secreted into the lumen; including acid, mucus, bicarbonate, and antibacterial substances (e.g., immunoglobulin's and lactoferrin) and the principal function of gastric acid is to reduce the number of ingested bacteria entering the small intestine (Wallace, Mcknight, & Brian, 2000).

The gastric mucus consists of a viscous, elastic, adherent and transparent gel secreted by apical expulsion from surface epithelial cells. It is fashioned by ~ 95% water and ~ 5% mucin glycol proteins that covers the entire gastrointestinal mucosa, and its luminal surface is coated with a film of surfactant phospholipids with strong hydrophobic properties. Bicarbonate is secreted by surface epithelial cells and its role is to neutralize acid diffusing into a stable, adherent mucus gel layer and to be quantitatively sufficient to maintain a near neutral pH (~ 7.0) at the mucus-mucosal surface interface. When the mucus bicarbonate barrier is overwhelmed or breaks down in different disease conditions, the next series of protective mechanisms come into play, including epithelial repair and maintenance and distribution of mucosal blood flow (Laine, Takeuchi, & Tarnawski, 2008).

Subsequent to mucus-bicarbonate "barrier", the next line of mucosal protection is formed by a continuous layer of surface epithelial cells, which secrete mucus and bicarbonate and generate

prostaglandins (PGs), heat shock proteins (HSP) and trefoil factor family peptides (TFFs). This epithelial barrier serves to separate the digestive lumen from the internal compartments of the organism. Its main role is to maintain a selective exchange of different substances (secretions, nutrients, etc.) between these two compartments, and to assure the protection of the organism against the penetration of micro-organisms and other exogenous antigens, essentially contained in food(Tulassay & Herszenyi, 2010).

PGs are produced by gastric mucosal epithelial cells from arachidonate metabolism through the action of cyclooxygenases (COX). The ability of exogenous PGs to prevent mucosal damage caused by corrosive substances such as absolute ethanol, concentrated bile or hyperosmolar solutions has been termed "cytoprotection" (Farhadi et al., 2003). Particularly prostaglandin E2 (PGE2) and prostacyclin (PGI2) have long been known to have "cytoprotective" effects on the gastrointestinal epithelium and therefore they can be vital for the maintenance of the gastric integrity and inhibition of their synthesis results in the reduction of gastric mucosal blood flow and gastric mucosal damage (Abdel-Salama, Czimmer, & Mozsik, 2001).

HSPs are generated by gastric epithelial cells and it is essential for maintenance of cellular homeostasis during normal cell growth and for survival during various cellular stresses, such as increased temperature, oxidative stress, and cytotoxic agents, preventing protein denaturation and protecting cells against injury. TFFs comprises a group of small peptides (6.5–12 kDa) secreted abundantly by surface epithelium, which has been demonstrated play an important role in mucosal integrity by inhibiting apoptosis and inflammation, and augmenting the barrier function of mucus (Taupin & Podolsky, 2003). They regulate re-epithelialization by stimulating cell migration and exert mucosal protective action from a broad range of toxic chemicals and drugs (Laine et al., 2008).

In addition, other mediators such as nitric oxide (NO), calcitonin gene related peptide (CGRP) as well as some hormones including gastrin and cholecystokinin (CCK), ghrelin, leptin and GRP protect gastric mucosa against damage induced by corrosive substances. This protective action has also been attributed in part to the release of PGs because it could be abolished by the

pretreatment with indomethacin and restored by the addition of exogenous PGE2 (Ashkan Farhadi, Banan, & Keshavarzian, 2003).

The gastric mucosal microcirculation plays crucial role in the maintenance of gastric integrity, especially for bringing oxygen and nutrients and removing toxic substances. Exposure of the gastric mucosa to an irritant or acid back diffusion occurrence leads to a marked increase in mucosal blood flow. This increase allows removal of the back-diffusing acid and noxious agents and it is essential for mucosal defence (Holzer, 2006; Laine et al., 2008).

1.3.2. Aggressive factor for pathogenesis of peptic ulcer disease

NSAIDs such as aspirin and indomethacin are effective as anti-inflammation, anti-rheumatics, antipyretics, platelet inhibition and analgesics. NSAIDs have direct cytotoxic effect on gastric mucosa cells since most of NSAIDs are weak organic acids and can freely pass through cellular membrane to intracellular compartment under acidic environment of the stomach and dissociate easily. The intracellular concentration of NSAIDs is much higher than the extracellular one (Scheiman, 1996).

NSAIDs cause mucosal erosion, ulceration and hemorrhage through inhibition of COX-1 and it decrease PGI2 and PGE2 that have mucosa protective effects, decline the blood flow in gastric mucosa, decrease the provision of oxygen and nutrition, slow the turnover of mucosa cells, lessen the syntheses and secretion of mucus, damage the mucus and mucosa barriers (Zarghi & Arfaei, 2011). And it brings uncoupling of mitochondrial oxidative phosphorylation. The consequences of such mitochondrial dysfunction are a decrease in adenine tri-phosphate (ATP) production and an increase in adenine mono-phosphate (AMP) and adenine di-phosphate (ADP) levels, which are then responsible for increments of intracellular calcium concentration. These changes are followed by mitochondrial injury, increased generation of reactive oxidative state (ROS) and alterations in the Na+/K+ balance, which lead to weakening of the mucosal barrier and cellular necrosis (Wallace et al., 2000).

It also inhibits gastric peroxidases and may increase mucosal hydrogen peroxide and hydroxyl ion levels that will cause oxidative mucosal damage. NSAIDs, particularly those of acidic nature, can

directly kill epithelial cells. Various mechanisms have been proposed for this cytotoxic action of NSAIDs, including the induction of osmotic lysis subsequent to trapping of charged NSAIDs with the epithelial cells and death of the epithelial cell (Adinortey, Ansah, & Galyuon, 2013). Furthermore, NSAIDs inhibit the expression of HSP induce the release of various inflammatory factors, such as Leukotriene B4 (LB4) and histamine, and finally damage capillary vascular, increase vascular permeability and reduce blood flow into the mucosa (Wallace, 1985).

H. pylori infection induces gastric inflammation, ulcer, and cancer through interrelated cascade of colonization in the host. Bacterium starts colonization by adapting the harsh acidic environment of the stomach since it has the capability to sense the pH of its surroundings and favor the movement towards the less acidic region through catalyzing urea hydrolysis with the formation of ammonium (NH3) that neutralizes the surrounding gastric acid and protects itself from the strong acidity of the stomach. It penetrates the mucus layer of stomach, adhere the surface of gastric mucosal epithelial cells, proliferate and finally form the infectious focus. The gastric lesion is developed by destruction of mucosa, inflammation and mucosal cell death (Chai, 2011).

Once the gastric mucosal barrier is weakened, pathogen uses adhesive molecules to adhere to the epithelial lining of the stomach and it establishes interaction with the host using several toxins that leads to development of inflammation or gastritis. Prolonged inflammation damages epithelial cells leading to ulcers in the stomach. Genetic changes in the host cell due to H. pylori infection leads to development of gastric cancer (Neelapu, Nammi, & Pasupuleti, 2014). H. pylori can also produce mucolytic enzyme that causes the gastric mucous degradation. The degraded mucus losses its viscosity and elasticity and crumbled mucus can provide as a nutrient to H. pylori thus, allows the diffusion of hydrogen ion back to the stomach wall and the erosion of mucosa layer. It also generate an extracellular protease which split the polymer of glycoprotein in gastric mucus and make it to wipe out easily to the gastric mucous barrier and allows the gastric epithelium to contact directly with attack factors such as gastric acid, pepsin, cholic acid and drugs (Cellia et al., 2009).

Alcohol absorption into the bloodstream occurs throughout the gastrointestinal tract and its direct contact with the mucosa can induce numerous metabolic and functional changes (Bode, 1997).

These alterations may lead to marked mucosal damage, which can result in a broad spectrum of acute and chronic diseases, such as gastrointestinal bleeding and ulcers alcoholic gastritis leads to the impairment of the integrity of gastric mucosal barrier, contributing to acid reflux into the subluminal layers of the mucosa and sub mucosa (Oh et al., 2005).

The decreased formation of prostaglandins might also play a role in alcohol-induced mucosal injury, alcohol-dependent increase in the production of leukotrienes also contribute to the development of alcohol-induced damage. It is important to emphasize that changes induced by short-term exposure to alcoholic beverages are rapidly reversible while prolonged alcohol exposure leads to progressive structural mucosal damage (Bode, 1997). It is also involved in the formation of oxidative stress and responsible for weakening of the mucosal barrier and cellular necrosis(Silva, Maria Izabel Gomes de Sousa, & Florenço, 2011).

Stimulation of gastric acid secretion by physiological stress increases susceptibility to gastro duodenal ulceration. It is also known to modify gastric blood flow, which plays an important role in the gastric mucosal barrier, and it affects possible mediators such as cytokines, Corticotropin-releasing hormone (CRH) and Thyrotrophic- releasing hormone (TRH). Furthermore, stress seems to have different effects on gastric motility including delayed gastric emptying, which could increase the risk of gastric ulcer, while accelerated emptying could increase the net acid load delivered to the duodenum, enhancing the risk of duodenal ulcer and also promote the growth of H. pylori (Susan Levenstein, Sigurd Ackerman, & Dubois, 1999).

Continued smoking augments the secretion of HCl and pepsin and is also expected to modify the contents of gastric juice and pepsin iso enzyme patterns. The increased gastric acid secretion is mediated through the stimulation of H2-receptor by histamine released after mast cell degranulation and due to the increase of the functional parietal cell volume. It also causes mucosal injury by increasing content of free oxygen radicals, pituitary vasopressin, gastric endothelin and pituitary vasopressin. Nicotine not only induce ulceration, but also potentiate ulceration caused by H. Pylori, alcohol, NSAID or cold restrain stress and also alter the processes important in gastric and duodenal mucosal integrity or protection such as mucosal bicarbonate secretion, prostaglandin content, mucosal blood flow or epidermal growth factor (Walker & Taylor, 1997).

1.4. Treatment of peptic ulcer disease

1.4.1. Conventional drugs

Proton pump inhibitors (PPIs) are the most potent suppressors of gastric acid secretion that inhibit gastric H+, K+-ATPase irreversibly and inactivating the pump molecule since they block the final step in acid production, PPIs are effective in acid suppression. Several PPIs are available for clinical use among them omeprazole, lansoprazole, dexlansoprazole,rabeprazole, esomeprazole and pantoprazole are common. Nausea, abdominal pain, constipation, flatulence, and diarrhea are most common side effects encountered in PPIs. Sub-acute myopathy, arthralgias, headaches, and skin rashes also have been reported. PPIs are metabolized by hepatic cytochrome (CYPs) and interfere with the elimination of other drugs cleared by this route (Goodman & Gilman's, 2011).

Long-term and/or high-dose administration of PPI has been reported to be associated with an increased risk of bone fracture and with increased susceptibility to certain infections (e.g., hospital-acquired pneumonia, community-acquired Clostridium difficile)(Gregory A. Coté, W., & Howden, 2008). Hyper gastrinemia is more frequent with chronic omeprazole administration and it may leads to rebound hyper secretion of gastric acid upon discontinuation of therapy and may also promote the growth of gastrointestinal tumors. In rats, long-term administration causes hyperplasia of ECL cell and the development of gastric carcinoid tumors (Goodman & Gilman's, 2011).

The H2 receptor antagonists (cimetidine, ranitidine, famotidine and nizatidine) inhibit acid production by reversibly competing with histamine for binding to H2 receptors on the basolateral membrane of parietal cells. These drugs are suppressing 24-hour gastric acid secretion by ~70%. Side effects such as diarrhea, headache, drowsiness, fatigue, muscular pain, and constipation may come across H2 receptor blocker. Drug interactions with H2 receptor antagonists occur mainly with cimetidine. It inhibits CYPs (e.g., CYP1A2, CYP2C9, and CYP2D6) and can increase the levels of a variety of drugs that are substrates for these enzymes(Goodman & Gilman's, 2011).

PGE2 and its analog misoprostol can prevent gastric injury by cytoprotective effects that include stimulation of mucin and bicarbonate secretion and increased mucosal blood flow (Wolfe &

sachs, 2000). Sucralfate is a complex sucrose salt in which hydroxyl groups have been substituted by Aluminum hydroxide and sulphate. Sucralfate may act by two mechanisms: In the gastric environment, aluminum hydroxide dissociates, leaving the polar sulphate anion, which can bind to positively charged tissue proteins found within the ulcer bed and providing a physicochemical barrier impeding further tissue injury by acid and pepsin(Dennis Kasper, Anthony Fauci, & Stephen Hauser, 2017).

Antacids are basic substances which neutralize gastric acid and raise pH of gastric contents to > 4. Peptic activity is indirectly reduced if the pH rises above 4, because optimum peptic activity is exerted between pH 2 to 4. They also increase tone of esophageal sphincter and reduce the reflux of the acid and gastric contents in to the esophagus. Hence, they are also useful in the treatment of gastro-esophageal reflux disease (GERD)(Gulia & Choudhary, 2011). It may affect a number of drugs by altering rates of dissolution and absorption, bioavailability, and renal elimination (e.g., thyroid hormones, allopurinol, and imidazole antifungal). Aluminum ions (Al3+) and Magnesium (Mg2+) antacids are also tendency to chelate with other drugs and they form insoluble complexes in the GI tract that affects the absorption of other drugs (Goodman & Gilman's, 2011).

The muscarinic (M1) receptor antagonist pirenzepine and telenzepine can reduce basal acid production by 40-50% through suppressing neural stimulation of acid production via actions on M1 receptors of intramural ganglia. Because of their relatively poor efficacy, significant and undesirable anti-cholinergic side effects, and risk of blood disorders (pirenzepine), they rarely are used today (Udaykumar, 2007).

Antimicrobial agents that are clinically effective against H.pylori are: amoxicillin, clarithromycin, tetracycline and metronidazole. First-line therapy is usually initiated with a PPI-based three-drug regimen for 10 to 14 days it contains PPI, amoxicillin and clarithromycin. If a second course of treatment is required, a four-drug regimen with a bismuth salt, metronidazole, tetracycline, and PPI should be used. However, any single drug is relatively ineffective because resistance develops rapidly, especially to metronidazole. Earlier, bismuth was used in the combination regimens for eradication of H.pylori but due to poor patient acceptability is infrequently used now (Dipiro JT et al., 2014)

1.4.2. Natural products

The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer. In this modern era also 75-80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in the herbal medicine or plant are part of the physiological functions of living flora and hence they are believed to have better compatibility with human body (Kamboj, 2000). Approximately 25% of modern medicines are directly descended from plants first used traditionally. Many others are synthetic analogues built from prototype compounds isolated from plants. Generally, 70% modern medicines are derived from natural products (Pathak & Das, 2013).

A growing number of plants have been also reported to have antiulcer activity. Among them *Osyris* quadripartite(Abebaw, Mishra, & Gelayee, 2017),*Trichosanthes cucumerina*(Arawwawala, Thabrew, & Arambewela, 2010), *Dodonaea viscose*(Ashkan Farhadi et al., 2003),*Basella rubra* (S. S. Deshpande, Shah, & Deshpande, 2003),*Clitorea ternatea* (Marhuenda, Martin, & Alarcon de la Lastra, 1993),*Cinnamomum tamala*(Eswaran et al., 2010), *Red pepper and garlic bulb* (Gehan Salah Eldin Moram, Abouzeid, & Salman, 2016), *Gynocardia odorata* (Hina et al., 2013),*Croton zambesicus* (Okokon & Nwafor, 2009),*Terminalia chebula* (Hawkey & Rampton, 1985), *Citrullus colocynthis* (Prasanth reddy et al., 2012),*Tephrosia purpurea* (Sabri Fatima Zohra, Belarbi Meriem, & Sabri Samira, 2012),*Salvadora indica* (Sahoo et al., 2016),*Momordica dioica* (Vijayakumar, 2011).

1.5. Experimental plant

Urtica simensis (U.simensis) belongs to the genus *Urtica* of the family *Urticaceae*. They are often easily recognizable particularly after having experienced the sting. It can be found in a variety of habitat and soil type. Different species of the plant occur as a perennial plant in temperate zones

of Africa, Asia, America and Europe. It is commonly found growing in rich soils in forest clearings, old fields and wasted places(Seifu, Mehari, Atlabachew, & Chandravanshi, 2017).

Several species of the genus *Urtica* (especially the *Urtica dioica* L.) are used medicinally to treat a variety of ailments. The *U. dioica* has been used for hundreds of years to treat rheumatism, arthritis, gout, eczema, anemia, urinary tract infections, kidney stones, hay fever and early stages of an enlarged prostate (called benign prostatic hyperplasia). The plant has antimicrobial activity against wide variety of bacteria and fungi organisms. Beyond its antimicrobial activity, the plant also has antioxidant, anti-inflammatory, antiulcer and analgesic effect (Gülçin, Küfrevioğlu, Oktay, & Büyükokuroğlu, 2004).

The plant *U. simensis* commonly known as Nattle (English), Sama (Amharic), Dobii (Oromifaa), and Ameie (Tigrigna)is one of species of Nettel and endemic in Ethiopia. It is dark green grows all around the year and leaves and young shoots are also eaten in times of famine in some areas of Ethiopia. Ecologically it is found in upland grassland areas most common in disturbed localities, often abundant near houses(Gebrehiwot & Hundera, 2014; Gebremedhin G & Kalayou, 2013).

Figure 1; Photograph of matured *U. simensis* at time of flowering.

The ranges of moisture, crude protein, crude fat, crude fiber and total ash in the leaves of *U. simensis* collected from three different areas (Debreberhan, Fitche and Ambo) were found to be 76.8-78.9 %, 25.1-26.3 %, 2.2-2.3 %, 8.5-9.4 % and 20.8-26.4 %, respectively and mineral contents of raw samples (iron, zinc and calcium) were 38.4-47.0, 2.87-5.8 and 768.6-793.4 mg/100g, respectively and they were found to be rich in vitamin C (82.65- 84.3%). Tannin ranged from 25.3-27.0 mg/100g while oxalate was 8.59- 9.33 mg/100g. The above finding show that these indigenous leafy vegetable has high nutritive contents and its consumption need to be promoted in the various regions of Ethiopia (Eskedar Getachew Assefa, Haki, & AddisDemoz, 2013).

Leaves of *U. simensis* have a substantial anti-oxidant activity ranging between 2.28-2.42 mg ascorbic acid equivalent/g of dried leaves. Total phenols ranged from 15.75 to 22.67 mg gallic acid equivalent/g of dried leaves. Total tannin content ranged between 0.496 to 1.54 mg gallicacid equivalent/g of dried leaves. Similarly, total flavonoids concentration varied between 6.89 to 9.03 mg catechin equivalent/g of dried leaves (Seifu et al., 2017).

As described in different ethno botanical studies U. *simensis* has been frequently used traditional medicine for various ailments like peptic ulcer disease, malaria(Wubetu, 2017), Rh factor, heart failure (Molla & Asfaw, 2014), gastritis, wound(Chekole, Asfaw, & Kelbessa, 2015).In addition, the plant is reported to be used for the management of acute stomachache, body swelling and gonorrhea(Getu Alemayehu, Zemede Asfaw, & Kelbessa, 2015).

The aqueous fraction of *U. simensis* showed anti-diabetic activity in a dose dependent manner(Tsegaye, Urga, & Asres, 2008). Butanol fraction of 80% methanol extracts of *U. simensis* exhibited greater activity against gram positive bacteria while ethyl acetate fraction revealed greater activity against gram negative bacteria and fungi (Kassa, 2016).

1.6. Rationale for the study

The incidence of PUD varies with the age, gender, geographical location and is associated with severe complications including hemorrhages, perforations, gastrointestinal obstruction, and

malignancy. Thus, this clinical condition represents a worldwide health problem because of its high morbidity, mortality and economic loss (Silva et al., 2011). It affects 4 million people worldwide annually. Worldwide prevalence of the disease is about 40% in the developed countries and 80% in the developing countries (Adinortey, Ansah, Galyuon, & Nyarko, 2013). An estimated 15000 deaths occur each year as a consequence of peptic ulcer. Annual incidence estimates of peptic ulcer hemorrhage and perforation were 19.4–57 and 3.8–14 per 100,000 individuals, respectively. The average 7-day recurrence of hemorrhage was 13.9% and the average long-term recurrence of perforation was 12.2%. The increasing incidence and prevalence of ulcers have been attributed to several factors encountered during day to day life, such as stress, exposure to bacterial infection, and use of NSAIDs (Dongo, Uhunmwagho, & Kesieme, 2017).

Pharmacological treatments are the mainstay treatment for peptic ulcer for many centuries. Despite the fact, most of drugs are associated with adverse reactions, requirement for multiple doses per day, large tablet size, and the need to separate the drug from meals and potentially interacting medications(Dipiro JT et al., 2014).

There are many plants in Ethiopia and throughout the world which are traditionally used for the treatment of peptic ulcer diseases among which *U. simensis* is one of them. Therefore, this study attempted to validate the traditional use of this endemic medicinal plant for anti-gastric ulcer activity and also aimed to provide a clue about the qualitative phytochemical constituents of the plant so as to get insight into the nature of phytochemicals responsible for its action. The finding of this research could be used as an input in searching of new drug with anti-gastric ulcer activity that might solve problems associated with the conventional drugs.

2. OBJECTIVES

2.1. General objective

- To evaluate the anti-gastric ulcer activity of aqueous (AQ) and 80% methanol (ME) leaf extracts of *Urtica simensis* in rats.

2.2. Specific objectives

- To evaluate acute toxicity profile of both AQ and 80% ME extracts of *U.simensis* leaf in rats.
- To evaluate AQ and 80% ME extract of *U. simensis* for their anti-secretory effect using pylorus ligation-induced gastric ulcer models in rats.
- To evaluate AQ and 80% ME extract of *U.simensis* for their gastro protective effect using indomethacin induced gastric ulcer models in rats.
- To evaluate AQ and 80% ME extract of *U. simensis* for their gastro-protective effect using ethanol induced gastric ulcer models in rats.
- To determine the phytochemical constituents present in AQ extract of the leaf *U. simensis*.
- To determine severity of ulceration among pylorus ligation, indomethacin and ethanol induced gastric ulcer models in rats.

3. MATERIALS AND METHODS

3.1. Drugs and chemical

All solvents used for the extraction process are of laboratory grade. Drugs and chemicals used in the study include: Distilled water (Ethiopian Pharmaceutical Manufacturing Factory, Ethiopia), methanol and chloroform (Research Lab Fine Industries, India), glacial acetic acid, ammonia, hydrochloric acid and ferric chloride (BDH Laboratory Supplies Poole, England), acetic anhydride and Mayer‟s reagent (May and Baker LTD Dagenham, England), and Dragendroff‟s reagent and sulfuric acid (Fisher Scientific,UK), Cimetidine (Addis pharmaceutical company, Ethiopia), Ethanol (Dallul pharmaceutical, Ethiopia), Indomethacin (Leben, Laboratories,India). Phenophthalein indicator (Goenka chemical industry, India), sodium hydroxide (BDH, chemical lab, England), Alcian Blue (Arnish, Laborates, India), Sucrose (JHD, China), Magnesium chloride (Carbo Erba, Italy), Diethyl ether (Lobal, chemi, India) were used in the study.

3.2. The plant material

Leaves of *U. simensis* were collected from Ayertena, kolfe keranio subcity, Addis Ababa on March 21, 2018. The plant was authenticated by a taxonomist and a voucher specimen (OA/001) was deposited at the National Herbarium of College of Natural and Computational Sciences, Addis Ababa University for future reference.

3.3. Experimental animals

Healthy wistar rats of either sex weighing about 150- 200 gm were used for the experiment. The rats were obtained from animal house of School of Pharmacy; Addis Ababa University (AAU).The animals were kept in cages at room temperature and on a 12/12 h light/dark cycle with access to pellet food and water *ad libitum*. Rats were acclimatized to laboratory condition for one week prior to the experiments. The care and handling was according to international guidelines for the use and maintenance of experimental animals (OECD, 2008).

3.4. Preparation and extraction of plant materials

After collection, the leaves were initially washed using running tap water to remove dirt or dust and dried under shade in pharmacology laboratory within the school of pharmacy. The leaves were then chopped into small pieces manually and ground into coarse powder mechanically using a clean mortar and pestle. The powder sample was weighed and stored in air tight containers until extraction commenced.

3.4.1. Preparation of methanol extract

The extraction was carried out by maceration technique using 80% ME as a solvent. Two hundred fifty gram of the dried powder was weighed using electronic digital balance and soaked in a clean flask containing 80% methanol in distilled water (200 g in 1600 ml) i.e. (1:8 (w/v). The plant material was macerated for 72 h with occasional shaking using mini orbital shaker (Bibby Scientific Limited Stone Staffo Reshire, UK) tuned to 120 rpm. The extract was filtered through double layered muslin cloth followed by whatman No.1 filter paper (Maidstone, UK). The marc was then re-macerated for a second and third time by adding another fresh solvent. The resultant filtrates were combined and concentrated using a rotary evaporator (Buchii model R-200, Switzerland) set at 40°C to remove methanol and finally the concentrated aqueous solution filtrate was placed in deep freezer set at -20 ºC to solidify and dried in a lyopholizer (Operan, Korea vacuum limited, Korea).

3.4.2. Preparation of aqueous extract

Two hundred gram of the dried powder was weighed and boiled with 2000 ml of distilled water for 30 min. The decoction was filtered twice with cotton gauze and aqueous solution filtrate was placed in deep freezer set at -20 ºC to solidify and dried in a lyopholizer.

3.5. Acute toxicity test

Acute oral toxicity test for both AQ and 80% ME extracts of the leaf *U.simensis* ware performed according to the OECD guideline 425(OECD, 2008). Each extracts (AQ and 80% ME) at a dose of 2000 mg/kg ware administered sequentially and rats were observed individually for behavioral

profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation, and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response, and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and for morbidity or mortality, after dosing continuously for 2 hours, periodically during the first 24 hours (with special attention given during the first 4 hours). Based on the results of the first rat, another 4 female rats were recruited and fasted for 24 h. Thereafter, they were given the same dose and were observed for any sign of toxicity or death in the next 14 days

3.6. Animal grouping and dosing

Rats were randomly divided into eight groups each comprising of six rats. Group I (negative control) was treated with distilled water, Group II (positive control) was treated with Cimetidine 100 mg/ kg, p.o (CI 100) , Group III- VIII were treated with different doses of AQ and 80% ME extracts [100 mg/kg (AQ100, ME 100), 200 mg/kg (AQ 200, ME 200) and 400 mg/kg (AQ400, ME400)]. Five days pretreatment study were done in indomethacin and ethanol induced ulcer model, rats were divided into three groups and treated with AQ200 (GI), ME200 (GII) and CI100 (GIII) respectively .The standard drugs and plant extracts were administered orally.

3.7. Pylorus ligation induced gastric ulcer

Animals were fasted for 48 hours before the study, but had free access to water. After 1 hour of drug treatment, they were anesthetized with ether and the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated and it was performed with caution to avoid traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by ether after 6 hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out, and the contents were drained into a glass tube. The volume of the gastric juice was measured after centrifugation at 2000 rpm for 10 minutes. From the supernatant, aliquots were taken for the determination of pH and total acidity. Each stomach was examined for lesions and indexed according to severity (Prasanth reddy et al., 2012).

3.8. Indomethacin-induced gastric ulcer

Gastric lesions were induced with indomethacin (40 mg/kg) administered to rats after fasting for 36 h. Animals were treated with either vehicle, extracts or standard cimetidine orally 30 min prior to induction of gastric lesions. The animals were sacrificed 5 h after treatment with the ulcerogenic agent to assess the ulcer score and mucous producing activity (Yesiladaa & Gurbuz, 2010).

3.9. Ethanol-induced gastric ulcer

All the animals were fasted for 48 hours before administration of ethanol. The gastric ulcers were induced in rats by administrating ethanol (90%) (1 ml/200 g) orally, after 60 minutes of extract & treatment. The animals were anesthetized 1 hour later with ether, and the stomach was incised along the greater curvature and ulceration was scored as for the pyloric ligation-induced ulcer model and gastric mucus was determined(Vijayakumar, 2011).

3.10. Determination of anti-ulcer activity

3.10.1. Evaluation of stomachs

The stomachs were opened along the greater curvature and rinsed with normal saline to remove gastric contents and blood clots and examined by a 10x magnifier lens to assess the formation of ulcers. The ulcer index can be measured using the following scores as described by Reddy et al (Prasanth reddy et al., 2012).

Normal colored stomach --------- (0)
Red coloration---------- (0.5)
Spot ulcer ---------- (1)
Hemorrhagic streak ---------- (1.5)
Deep ulcers---------- (2)
Perforation---------- (3)

Ulcer index (UI) = UN + US + (UP/10)

Were UI=ulcer index, UN = average number of ulcer per animal, US = average of severity score And UP = percentage of animals with ulcer.

And percentage protective ratio was calculated as follow

% of protection= $100-\frac{[UIPreteated]}{[UIControl]} \times 100$(Adinortey, Ansah, & Galyuon, 2013).

3.10.2. Determination of pH

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water and pH of the solution was measured using pH meter (Abebaw et al., 2017).

3.10.3 Determination of total acidity

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water and was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was observed. The volume of 0.01N NaOH consumed was noted. The total acidity was expressed as mEq/L and calculated by the following formula (Abebaw et al., 2017).

Acidity = $\frac{\text{V NaOH X N X 100 mEq/L}}{0.1}$where V is volume and N is normality

3.10.4. Determination of gastric mucus

The glandular portion of gastric tissues was immediately transferred to 0.1% alcian blue solution prepared in 0.16 M sucrose and 0.05 M sodium acetate (pH 5.8) and stained for 2 h at room temperature. After the segments were rinsed twice with 0.25 M sucrose solution for 15 and 45 min, the dye complexed with the gastric mucus was extracted with 0.5 M magnesium chloride solution for 2 h (during this period the soaked stomach shaken for one min every 30 min). The extract was then mixed with equal volume of diethyl ether and centrifuged at 3000 rpm for 15 min. Absorbance was determined at 580 nm after decanting the lower layer, using spectrophotometer. Mucus amount were calculated using standard curves of alcian Blue (Fig 2) (Baggio et al., 2007). And the fitted equation for the linear calibration curve was Y = 0.119 x+0.00 where represents the dependent variable (absorbance) and X stands for the independent variable (concentration of Alcian blue in microgram/ml). Thus, the absorbance measured in the

different treatment groups was used to get the corresponding concentration of Alcian blue which was complexed with the mucin on the wall of the glandular portion of the stomach.

Mucin content = $\frac{Alcianblue\ (\frac{\mu g}{ml})}{glandulartissue(g)}$(Gehan Salah Eldin Moram et al., 2016).

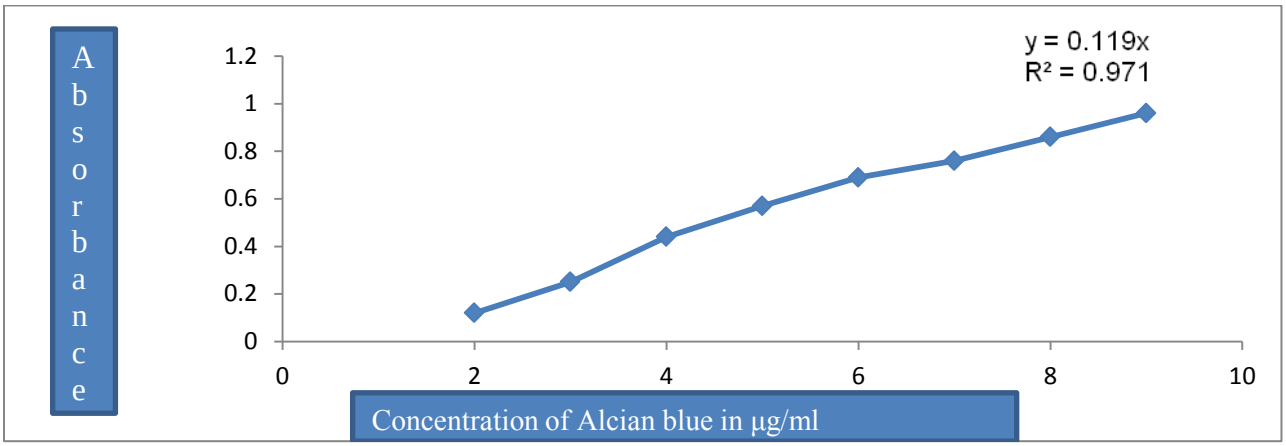

Figure 2; Calibration curve for alcian blue 8GX in aqueous solution

3.11. Preliminary photochemical screening

The qualitative photochemical investigations of AQ extracts of leaf of *U. simensis* were carried out using standard tests

Test for terpenoids

To 0.25 g of AQE, 2 ml of chloroform was added. Then, 3ml concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids (Pritish Shrestha, Sandeep Adhikari, Basanta Lamichhane, & Shrestha, 2015).

Test for saponins

To 0.25 g of AQE, 5 ml of distilled water was added in a test tube. Then, the solution was shaken vigorously and observed for a stable persistent froth. Formation of froth indicates the presence of saponins (Sabri Fatima Zohra et al., 2012).

Test for tannins

About 0.25 g of AQE was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added to the filtrate. A brownish green or a blue-black precipitate indicated the presence of tannins (Pritish Shrestha et al., 2015).

Test for flavonoids

About 10 ml of ethyl acetate was added to 0.25 g of AQE and heated on a water bath for 3 min. The mixture was cooled and filtered. Then, about 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonial layer indicated the presence of flavonoids (Ayoola et al., 2008).

Test for cardiac glycosides

To 0.25 g of AQE diluted to 5 ml in water2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underplayed with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxy-sugar characteristic of cardenolides (Ayoola et al., 2008).

Test for steroids

Mixing the extract with 2ml of chloroform then 2ml of each of concentrated H2SO4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids (Pritish Shrestha et al., 2015).

Test for alkaloids

A 0.25 g of AQE was diluted to 10 ml with acid alcohol, boiled, and filtered. To 5 ml of the filtrate, 2 ml of dilute ammonia and 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer‟s reagent was added to one portion and Dragendorff‟s reagent to the other. The formation of a cream (with Mayer‟s reagent) or reddish brown precipitate (with Dragendorff‟s reagent) was regarded as positive for the presence of alkaloids(Ayoola et al., 2008).

Test for Anthroquinones

A 0.25 g of the AQE was boiled with 10 ml of H2SO4 and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes(Ayoola et al., 2008).

Test for Phenols

About 0.25 g of AQE was treated with few drops of 5% neutral ferric chloride solution; the appearance of a greenish color indicated the presence of phenols(Pritish Shrestha et al., 2015).

3.12. Data analysis

Results are expressed as mean ± standard error of the mean (SEM). The experimental results were analyzed using the software Statistical Package for Social Sciences (SPSS), version 21. Statistical significance was determined by one way analysis of variance (ANOVA) followed by Turkey post Hoc test where P-value of less than 0.05 was considered statistically significant. Coefficient of determination (R^2), using linear regression analysis, was determined where appropriate. The analyzed data were then presented using tables and figure.

4. RESULTS

4.1. Acute toxicity test

Both 80 % MEQ and AQE of the leaves of *U.simensis* produced neither overt toxicity nor death during the 14 days observation period following oral administration of a single dose of 2000 mg/kg. In addition, neither food nor water intake was found to be reduced during the period.

4.2. Effects of the leaf extract on pylorus ligation-induced ulcer

As shown in table 1- AQ 200 & ME 200 ($p<0.05$), AQ 400($P<0.01$) and ME400 & CI 100 mg/kg ($p<0.001$) were exhibited significant rise in pH when compared to the negative control.

Both extracts at 100mg/kg dose did not show significant rise in pH of gastric secretion compared to the negative control ($p>0.05$).All doses of extracts except AQ100 & ME 100 ($p>0.05$) were exhibited significant reduction in total acidity ($p<0.001$) when compared to the negative control.

ME200, ME400 & CI100 were exhibit significant reduction in total acidity ($p<0.05$) when compared to the AQ100 & ME100.

All doses of the extract were exhibited significant reduction in volume of gastric secretion ($p<0.001$) when compared with negative control whereas AQ 200 & ME 200 ($p<0.05$), AQ 400, ME 400 & CI 100 ($p<0.001$) showed a significant reduction in the volume of gastric secretion as compared to AQ100 and ME100.

Concerning ulcer index and percentage of protection AQ 200 ($p< 0.05$), ME 200 ($p<0.01$), AQ 400 & CI 100 ($p<0.001$) showed significant reduction in ulcer index as compared to the control group. It was showing protection index of 33.7% (AQ100), 52.5% (AQ200), 65.3% (AQ400), 34.8% (ME100), 55.3% (ME200), 67 % (ME400) and 69.5% (CI 100) as compared with negative control (Table 1).

Table 1; Effect of aqueous and 80% methanol extracts of *U. simensis* or cimetidine on pH, Total acidity, gastric juice volume, ulcer index and percentage of protection in pylorus ligation-induced ulcer

Groups	PH	Total Acidity	Volume	UI	% protection
NC	1.79±0.14	83.16±2.28	8.7±0.25	23.5±1.01	
AQ100	2.56±0.16	64.91±3.05	6.5±0.31 ***a3***	15.58±0.71	33.7
AQ200	3.41±0.20 ***a1***	47.25±4.56 ***a3***	4.6±0.45 ***a3b1c1***	11.16 ±2.25 ***a1***	52.5
AQ400	3.64±0.32 ***a2***	44.38±6.32 **a3**	3.9±0.57 **a3b3c2**	8.16 ±2.59 ***a3***	65.3
M100	2.79±0.15	64.25±2.44	6.3±0.16 ***a2***	15.33±0.81	34.8
M200	3.50±0.28 ***a1***	45.4±6.43 ***a3b1c1***	4.4±0.60 ***a3b1c1***	10.5±3.30 ***a2***	55.3
M400	3.80±0.29 ***a3***	43.8±6.79 ***a3b1c1***	3.8±0.49 ***a3b3c3***	7.75±3.40 ***a3***	67.0
CI100	3.88±0.31 ***a3***	42.16±6.29***a3b1c1***	3.5±0.29 ***a3b3c3***	7.16±3.22 ***a3***	69.5

AQ= Aqueous extract, ME=80% methanol extract, Cimetidine =CI

Data are expressed as mean ± SEM (n=6); analysis was performed with One-Way ANOVA followed by Tukey test; **a** compared to negative control; **b** compared to Aqueous 100 mg/kg **c** compared to methanol 100 mg/kg ; 1: $p<0.05$, 2: $p< 0.01$, 3: $p< 0.001$

4.3. Effects of the leaf extract on Indomethacin induced ulcer

As depicted in Table 2, All doses of extracts produced a statistically significant alteration in mucin content compared to control groups such as AQ 100($P<0.05$), AQ 200,AQ 400,ME 100,ME 200,ME 400 and CI 100 ($P<0.001$). The percentage of increment in mucin content were 20.6 (AQ100), 51.8 (AQ200), 62.5(AQ400), 28(ME100), 55.5(ME200) and 64 (ME400) and the standard drug CI 100 (65.2) was comparable with the maximal dose (400mg/kg) of both extracts and there was a dose-dependent increment in gastric wall mucus in AQ (R^2 =0.93, $p<0.001$) and 80ME (R^2=0.92; $p<0.001$).

Indomethacin produced massive gastric ulcers in all rats, ulcers were mostly superficial and few were penetrating. Ulcer index was 28 ± 1.97 in ulcerative rat. Pretreatment of rats with AQ (R^2 =0.999, p<0.001) and ME (R^2 =0.98, p<0.001) extracts of *U. simensis* exhibited dose dependent reduction in ulcer index. AQ extract at different doses exhibited reduction in UI such as 100 (18.91 ± 0.89), 200 (14.83 ± 0.9), 400 (11.08 ± 3.18) and ME 100, ME 200 and ME 400 gave an UI of 18.41 ± 0.59, 13.41 ± 0.3 and 10.58 ± 3.41 respectively and CI 100 produced an UI 9.75 ± 3.14.Percentage of protection for different doses of extracts were AQ 100(32.5%), AQ200(47%), AQ400(60.4%), ME100 (34.25%), ME200(52.1%), ME 400 (62.2%) and CI 100(65.1%).

Table 2; Effect of the aqueous and 80 % methanol extracts of *U. simensis* on mucin content (µg/g), percentage of increment in mucin, ulcer index and percentage of protection on Indomethacin induced gastric ulcer in rats.

	Mucin content (µg/g)	**% of increment in mucin**	**UI**	**% of protection**
Control	32.68 ±0.52		28 ± 1.97	
AQ 100	41.16 ± 1.37^{a1}	20.6	18.91±0.89^{a1}	32.5
AQ 200	67.9 ± 0.69 ^{a3b3c3}	51.8	14.83 ± 0.9 ^{a3}	47
AQ 400	87.23 ± 2.11 ^{a3b3c3}	62.5	11.08 ± 3.18 ^{a3}	60.4
ME 100	45.38 ± 1.18 ^{a3}	28	18.41 ± 0.59 ^{a1}	34.25
ME 200	73.46 ± 1.07^{a3b3c3}	55.5	13.41 ± 0.3^{a3}	52.1
ME 400	90.94 ± 1.31 ^{a3b3c3}	64	10.58 ± 3.41^{a3}	62.2
C100mg/kg	93.87 ± 1.58 ^{a3b3c3}	65.2	9.75 ± 3.14^{a3}	65.1

AQ= Aqueous extract, ME=80% methanol extract, Cimetidine =CI

Data are expressed as mean ± SEM (n=6); analysis was performed with One-Way ANOVA followed by Tukey test; **a** compared to negative control; **b** compared to Aqueous 100 mg/kg **c** compared to methanol 100 mg/kg ; 1: p<0.05, 2: p< 0.01, 3: p< 0.001

As shown in table 3, there were remarkable changes in the gastric parameters of extract-treated group for five days as compared to ulcerated control. Significant reduction in ulcer index and increment in mucin content were exhibited in both AQ and ME extracts as well as the standard drug CI (P<0.001). The percentage of increment in mucin content were (74.8%) AQ200, (75.1%) ME200and its increment was comparable with the standard CI 100(75.9%).

Table 3;Effect of repeated (5days) treatment aqueous and 80 % methanol extracts of *U. simensis* and cimetidine on mucin content (µg/g), percentage of increment in mucin, ulcer index and percentage of protection on Indomethacin induced gastric ulcer in rats.

	Mucin content (µg/g)	**% of increment in mucin**	**Ulcer Index (UI)**	**% of protection**
Control	32.68 ± 0.52		28 ± 1.97	
AQ 200	129.6 ± 1.94^{a3}	74.8	7.5 ± 3.36 ^{a3}	73.21
ME 200	131.2 ± 1.94^{a3}	75.1	7.17 ± 3.22 ^{a3}	74.4
C 100mg/kg	135.6 ± 2.38^{a3}	75.9	6.83 ± 3.05 ^{a3}	75.6

AQ= Aqueous extract, ME=80% methanol extract, Cimetidine =CI

Data are expressed as mean ± SEM (n=6); analysis was performed with One-Way ANOVA followed by Tukey test; **a** compared to negative control; 1: p<0.05, 2: p< 0.01, 3: p< 0.001

4.4 Effects of the leaf extract on ethanol-induced ulcer

Oral administration of ethanol significantly decreased gastric wall mucus in the control rats when compared with different doses of extract (P<0.001), as shown in table 4, all doses of extracts showed significant increments in gastric wall mucus from 46.66 ± 0.96 to 75.87 ± 1.52 and the standard drug CI100 showed mucus content as 77.23 ± 0.64 µg alcian blue/g wet stomach. Dose dependent increment in mucin content was found with both AQ (R^2=1, P<0.001) and ME (R^2=1, P<0.001) extracts.

Table 4; Effect of aqueous and 80 % methanol extracts of *U. simensis* and cimetidine on mucin content (µg/g), percentage of increment in mucin, ulcer index and percentage of protection on ethanol induced gastric ulcer in rats

	Mucin content((µg/g)	% of increment in mucin	Ulcer Index (UI)	% of protection
Control	37.51± 0.89		34.33±1.33	
AQ 100	46.66± 0.96	19.6	24.91±1.47	27.44
AQ 200	60.64± 2.17 ^{a2}	38.1	18.33±1.23 ^{a2}	46.6
AQ 400	75.08±1.36 ^{a3}	50	14.91± 3.13 ^{a3}	56.5
ME 100	47.34± 1.14 ^{a2}	20.8	25± 1.07	27.2
ME 200	61.27±1.92 ^{a3}	38.8	16.75± 1.37 ^{a2}	51.2
ME 400	75.87 ± 1.52 ^{a3}	50.6	14.16 ± 4.50 ^{a3}	58.7
CI 100mg/kg	77.23± 0.64 ^{a3}	51.43	13.16± 4.2 ^{a3}	61.6

AQ= Aqueous extract, ME=80% methanol extract, Cimetidine =CI

Data are expressed as mean ± SEM (n=6); analysis was performed with One-Way ANOVA followed by Tukey test; **a** compared to negative control; **b** compared to Aqueous 100 mg/kg **c** compared to methanol 100 mg/kg ; 1: p<0.05, 2: p< 0.01, 3: p< 0.001

Administration of ethanol (1ml/200g) produced superficial or deep erosions and bleeding as examined in ulcerative rats at which the ulcer index was 34.33±1.33, were as pretreatment with extracts produced reduction in ulcer index ranges from 24.91±1.47 with AQ100 to 14.16 ± 4.5 with ME 400. Dose-dependent ulcer inhibition was examined with AQ ($R^2 = 0.968, P < 0.001$) and ME ($R^2 = 0.916, P < 0.001$) extracts against ethanol-induced ulcers in rat. The percentage of reduction in ulcer index was found to be 19.6% (p<0.001), 38.1% (p<0.001), and 50% (p<0.001) at doses of 100 mg/kg, 200 mg/kg, 400 mg/kg of AQ extract respectively and ME extracts also produced significant reduction in ulcer index (P<0.001) at doses of 100mg/kg (20.8%), 200mg/kg (38.8%) and 400mg/kg (50.6%).

Five days repeated dose study revealed that mucin contents were increased from 37.51 ± 0.89 for negative control to AQ 200 (101.95 ± 1.64), ME 200 (106.85 ± 1.87) & CI 100 (108 ± 2.11) and ulcer index was reduced from 34.33 ± 1.33 (negative control) to 9.5 ± 3.01(AQ200), 9.33 ± 2.95 (ME 200) and 9.16 ± 2.9(CI 100). Percentage of increments in mucin contents for extracts and standard were AQ 200 (63.2%), ME 200 (64.9%) and CI 100 (65.3%).

Table 5; Effect of repeated (5days) treatment with aqueous and 80 % methanol extracts of *U. simensis* and cimetidine on mucin content (µg/g), percentage of increment in mucin, ulcer index and percentage of protection on ethanol induced gastric ulcer

	Mucin content	**% of increment in mucin**	**Ulcer Index (UI)**	**% of protection**
Control	37.51± 0.89		34.33±1.33	
AQ 200	101.95 ± 1.64	63.2 **a3**	9.5 ± 3.01 **a3**	72.3
ME 200	106.85 ± 1.87	64.9**a3**	9.33±2.95 **a3**	72.8
CI100mg/kg	108 ± 2.11	65.3**a3**	9.16 ± 2.90 **a3**	73.3

AQ= Aqueous extract, ME=80% methanol extract, Cimetidine =CI

Data are expressed as mean ± SEM (n=6); analysis was performed with One-Way ANOVA followed by Tukey test; **a** compared to negative control; 1: p<0.05, 2: p< 0.01, 3: p< 0.001

More ulceration was exhibited in ethanol induced ulcer model when compared with indomethacin and pylorus ligation induced ulcer. As shown in table 6, Ulcer index of ulcerative control rats in those three ulcer induction model were 34.33 ± 1.33 (ethanol), 28 ± 1.97 (indomethacin) and 23.5±1.01 (pylorus ligation).

Table 6; Effect of different doses of aqueous and 80 % methanol extracts of *U. simensis* and cimetidine on ulcer index in Pylorus ligation induced ulcer, Indomethacin and Ethanol induced gastric ulcer in rats.

Treatments		Ulcer Index		
		Pylorus ligation	**Indomethacin**	**Ethanol**
NC		23.5±1.01	28 ± 1.97	34.33 ± 1.33
100 mg/kg	AQ	15.58±0.71	18.91±0.89	24.91 ± 1.47
	ME	15.33±0.81	18.41 ± 0.59	25 ± 1.07
200 mg/kg	AQ	11.16 ±2.25	14.83 ± 0.9	18.33 ± 1.23
	ME	10.5±3.30	13.41 ± 0.3	16.75 ±1.37
400 mg/kg	AQ	8.16 ±2.59	11.08 ± 3.18	14.91 ± 3.13
	ME	7.75±3.40	13.41 ± 0.3	14.16 ± 4.5
CI 100		7.16±3.22	9.75 ± 3.14	13.16 ±4.2

AQ= Aqueous extract, ME=80% methanol extract, Cimetidine =CI

4.5. Phytochemical screening

As shown in Table 7, alkaloids, terpenioids, tannins, saponins, phenols, and flavonoids were present in AQ extracts of *U.simensis.*

Table 7; Preliminary phytochemical screening of aqueous extracts of *U. simensis* leaf

Chemical constituent	**Aqueous extract (AQ)**
Alkaloid	+
Flavonoids	+
Tannins	+
Phenols	+
Saponins	+
Steroids	-
Terpenioids	+
Anthroquinones	-

Symbol indicates (+) present and (-) absence.

5. DISCUSSION

Medicinal plants, although assumed to be safe, are potentially toxic which necessitates investigation of their safety status (Ifeoma & Oluwakanyinsola, 2013). It is therefore important to properly evaluate their safety and efficacy profile of plants that are under use in traditional medicines.

Acute toxicity study revealed that both types of extracts (AQ & ME) at the dose of 2000mg/kg didn't exhibit any sign of toxicity or mortality up to 14 days and this finding suggested that both 80ME & AQ extracts have a wider safety margin and any substance that is not toxic at 2000mg/kg is considered relatively safe (Lorke, 1983).

The need for phytochemical screening has become imperative, since many plants accumulate biologically active, complex organic chemicals (secondary plant metabolites) in their tissues. In the present study, the preliminary phytochemical screening of AQ extracts of *U. simensis* revealed the presence of alkaloids, terpenioids, tannins, saponins, phenols, and flavonoids, those constituents are also present in 80 % methanol extracts (Kassa, 2016). These similarities in phytoconstituents may be responsible for comparable anti-ulcer activity of both types of extracts in all three models.

In the present study, three different doses of both AQ and 80% ME extracts (100, 200, and 400 mg/kg) were evaluated for their effect on different parameter such as volume of gastric secretion, pH, total acidity, ulcer index and mucin content along with the standard drug cimetidine (100 mg/kg). Severity of ulceration in pylorus ligation, indomethacin and ethanol induced ulcer model was evaluated with ulcer index and greater ulceration was found in ethanol induced model when compared with other two models.

It may be due to the ability of alcohol to directly induce severe lesion (deep and perforation) by penetrating the gastric mucosa and causing the cellular damage which in turn increase the permeability to sodium and water (Dwivedi, Chander, & Narayan, 2014), but in case of indomethacin and pylorus ligation mucosal damage is secondary to some factor such as the

reduction of endogenous prostaglandin synthesis which is known to be cytoprotective in the gastric mucosa due to indomethacin (Goodman & Gilman's, 2011)and in pylorus ligation the cause of gastric ulcer is due to stress induced increase in gastric acid secretion and these acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid (Deshpande, Shah, & Parmar, 2003).

Pylorus ligation is an important procedure that shows the possible changes in the parameters for gastric content e.g. volume of gastric juice, total acidity and pH (Lakshmi et al., 2010). Ulcers caused by pyloric ligation are due to increased accumulation of gastric acid and pepsin, leading to the auto digestion of gastric mucosa (Eswaran et al., 2010). Inhibition of gastric acidity is one of the important protective factors, since overwhelming of the mucosal defense mechanisms by acid level leads to ulcer formation(Arawwawala et al., 2010). This model is best to assess the potential parameter important to measure an overall anti gastric ulcer activity of extracts such as pH, total acidity, volume, ulcer index and percentage of protection and all of them were examined in this study. In this model AQ100 & ME 100 did not show statistically significant rise in pH of gastric juice and reduction in total acidity ($p>0.05$) when compared to the negative control, AQ 200 & ME 200 exhibit significant rise in pH and reduction of total acidity and maximal dose of extracts (AQ 400 & ME 400) were exhibited better neutralizing capacity ($p<0.001$) and it is comparable with standard CI 100.This indicates that the low dose of the extract is not an adequate dose to neutralize the acid when compared to medium and maximal dose and this finding is parallel with many studies (Hina et al., 2013; Prasanth reddy et al., 2012; Raju, Ilango, & Chitra, 2009).

Both AQ ($R^2 = 0.998, P < 0.001$) and ME ($R^2 = 0.976, P < 0.001$) extracts revealed dose dependent reduction in ulcer index. These results suggest that the extracts interfered with digestive effect of accumulated gastric juice and also possessed reduction of both gastric acidity and gastric secretory volume which could be partly attributed to its flavonoid component which decrease histamine secretion from mast cells by inhibition of histidine decarboxylase and blocked acid formation in parietal cells in response to histamine. H+ / K+ - ATPase, the gastric proton pump are also inhibited by flavonoids (Borrelli & Izzo, 2000). Alkaloidal constituents of both types of extract might also contribute to anti-secretory effect by increasing gastrointestinal pH and/or by H2-receptor antagonism. Another possible mechanism of alkaloidal constituents

whereby they produce anti-secretory effect may be an anti-cholinergic action evoked by muscarinic receptor blockers resulting in an inhibition of vagus stimulation (Mossadic, Khandker, & Faisal, 2000).

Indomethacin is a commonly used type of NSAIDs in animal experiment to induce gastric ulcer, dose dependent and repeated dose study ware employed to examine gastric cytoprotective effect of extracts and the results of single dose study showed doses dependent decrease in ulcer index and increase in content of mucin in both extracts (AQ & ME $p<0.001$) as well as the standard (CI $p<0.001$) in comparison to ulcerated control group.

The repeated-dose study showed a significant reduction in ulcer index ($P<0.001$) and increments in mucin contents ($P<0.001$) when compared with single dose of AQ200 & ME 200. Percentage of ulcer inhibition for repeated AQ 200 (72.3%) and ME 200 (72.8%) exhibited significant reduction when compared to the single-dose study for AQ200 (46.6%) and ME200 (51.2%). This suggests that the cumulative ulcer healing effect of the extract is better than that of the single dose. This finding signifies that the extract possesses a gastro protective effect in both dose dependent and time dependent manner.

Several scientific studies revealed that the phytoconstituents like flavonoids, tannins, terpenoids and saponins are responsible for gastro protective effects of extracts (Borrelli & Izzo, 2000). Flavonoids are effective to stimulate PGE2 production in gastric mucosal cells. Exogenous prostaglandins particularly E series protects gastro intestinal mucosa from the damage induced by a wide range of irritants and there is evidence that endogenous prostaglandins are important in maintaining gastro duodenal integrity (Okokon & Nwafor, 2009). Saponins and triterpenoids have been reported to have antiulcer activity in several experimental models by the formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively increasing prostaglandin level (Curtis, Wallace, & MacNaughton, 1995; Lewis & Hanson, 1991) Also, Tannins possess as an antiulcer agent by its astringency property and vasoconstriction effects. Due to precipitation of micro proteins on the ulcer site, a protective layer is formed which hinders gut secretions and protects the mucosa from toxins and other irritants.

Alkaloids have been reported to have antiulcer properties although the mechanism by which they reduce ulcer formation is not known. However, it is believed that one of the possible mechanisms is by activation of cyclooxygenase enzyme, which subsequently stimulates PG synthesis. Increasing bicarbonate level in the gastrointestinal lumen could be another possibility (Mossadic et al., 2000). Since both AQ and ME extracts contain the above phytoconstituents it might be responsible for the observed cytoprotective activity.

The result of ethanol induced ulcer model shown that both extracts (AQ & ME) at dose of 200mg/kg ($p<0.01$) and 400mg/kg ($p<0.001$) possess significant inhibition of ulcer index when compared with ulcerative control. This reveals that protection of ethanol induced ulceration needs increment in dose since ethanol produced more ulceration than other two model. The repeated-dose (7 days) study for ethanol-induced ulcer model showed a significant reduction in ulcer index ($P<0.001$) and increment in mucin content when compared with single dose study. This finding signifies that the extract possesses significant gastro protective effect in frequent administration when compared with single dose. Both extracts possess dose dependent antiulcer activity, this may be due to presence of flavonoids, tannins, and terpenoids. These active compounds have ability to stimulate mucus, bicarbonate and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Antioxidants consist of polyphenols, flavonoids that have the capability to neutralize unstable molecules called free radicals. Antioxidants disrupt the chain reaction in which free radicals turn other molecules into free radicals like themselves, a process of chain breaking or stabilization (Varga, 2000).

6. CONCLUSION

This study revealed that both aqueous and 80% methanol leaf extracts of *U.simensis* is endowed with a promising dose dependent anti-gastric ulcer activity in pylorus ligation, indomethacin and ethanol induced gastric ulcer model in rats. Their antiulcer activities may be related to anti-secretory as well as cytoprotective activities of one or more of the identified phytoconstituents in *U.simensis* such as alkaloids, terpenioids, tannins, saponins, phenols, and flavonoids that act either individually or in synergy to bring about the overall antiulcer effect. These findings provide a scientific support for folkloric use of *U.simensis* leaves as treatment of gastric ulcer. Based on these findings, it can be assumed that *U.simensis* could be a potential source for discovery of new and effective anti-gastric ulcer drug.

7. RECOMMENDATION

The following investigations are suggested for further study

- Fractionation of the crude extract and study the anti-ulcer activity.
- Further studies should be done to isolate, purify and identify pharmacologically active principle (s) responsible for the antiulcer activities of the plant.
- Further studies shall focus on elucidating mechanisms of action.
- The antiulcer activities of this plant should also be done in other models including in vitro evaluation of anti H. pylori.

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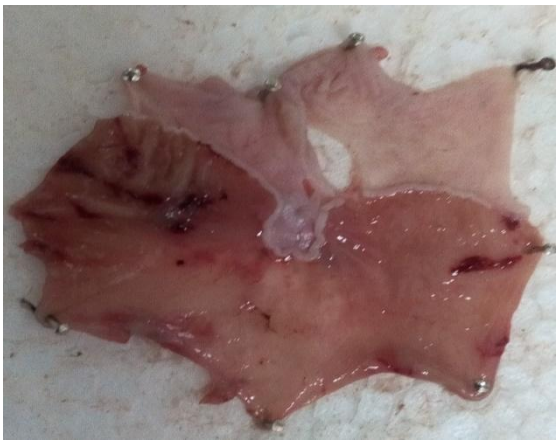
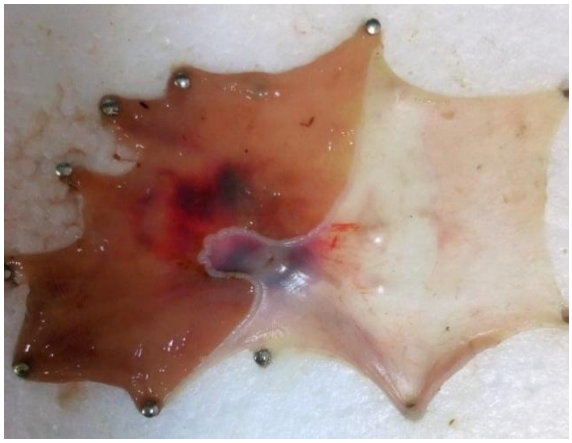
ANNEX I

Effect of different doses of aqueous and methanol extracts of *U.simensis* in pylorus ligation induced ulcer rats



Negative control



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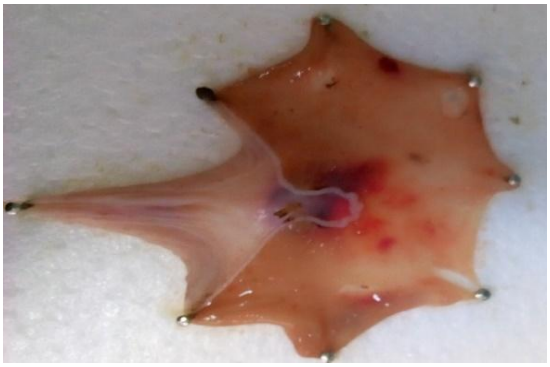

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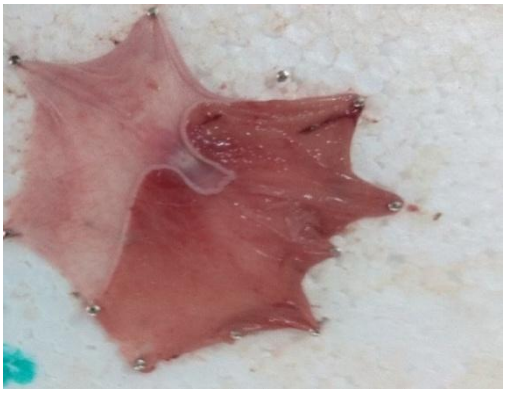

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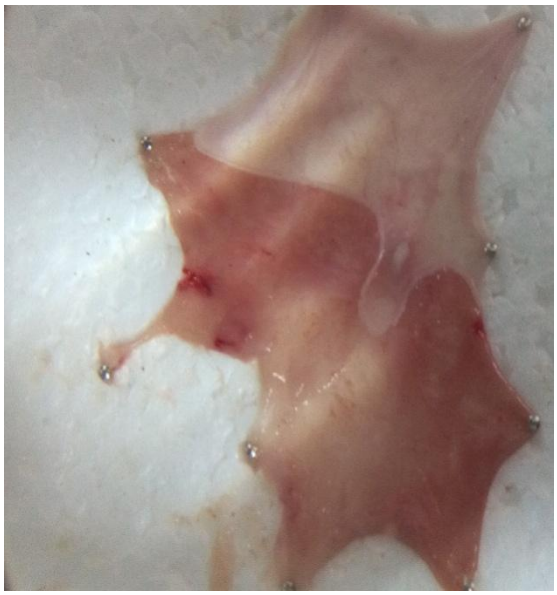

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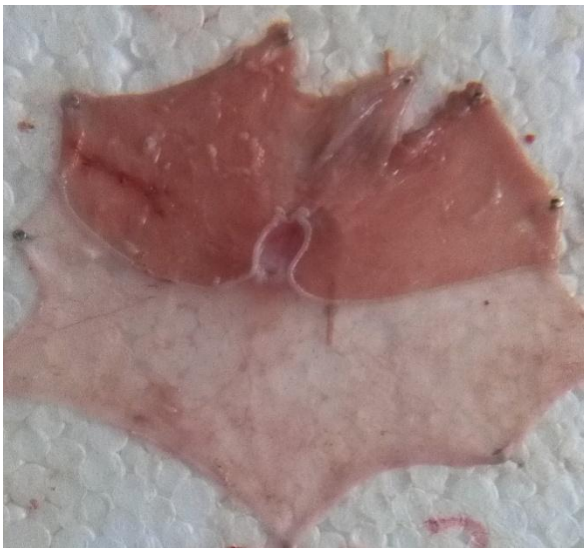

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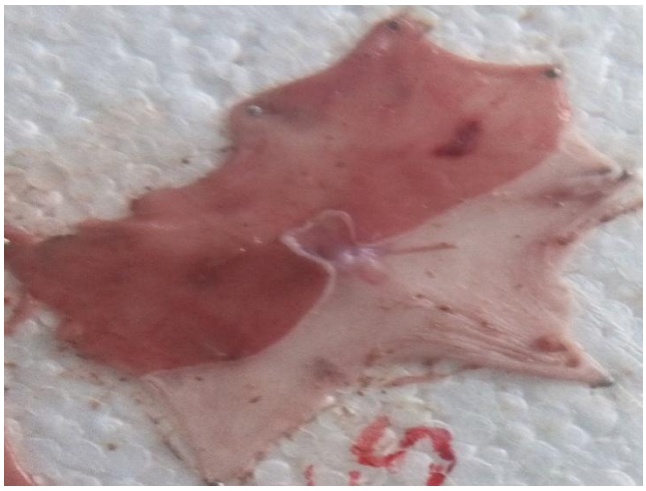

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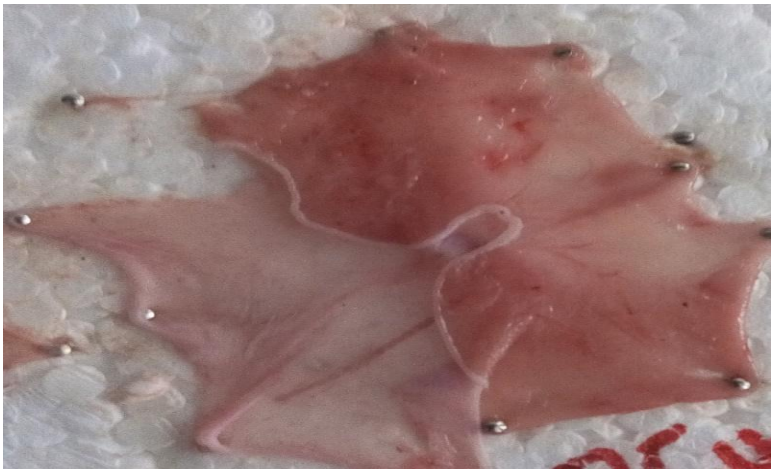
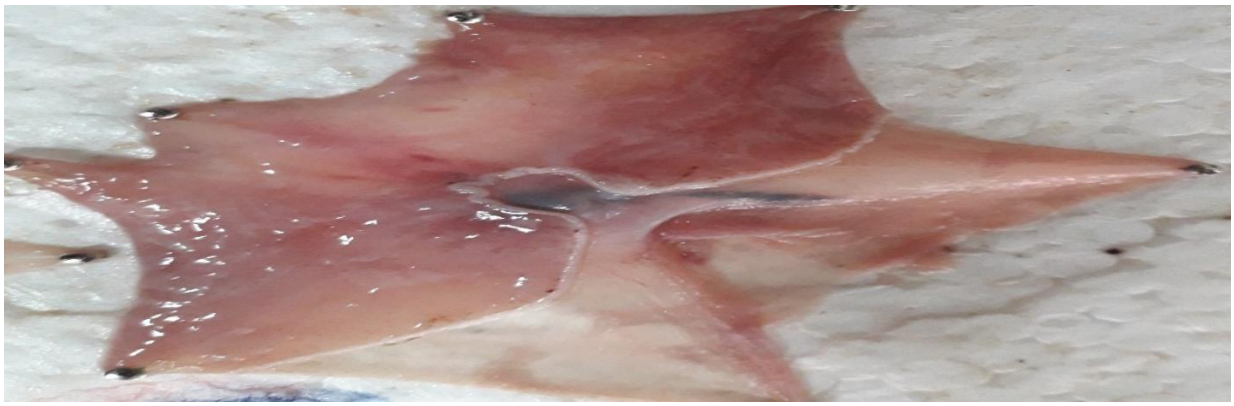


CI 100

ANNEX **II**

5 days Pretreatment of extract and standard on indomethacin induced ulcer model



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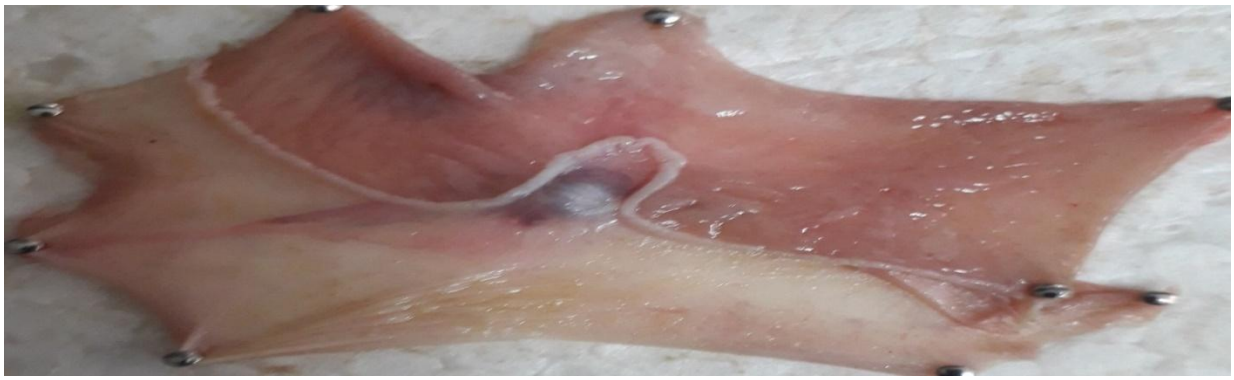

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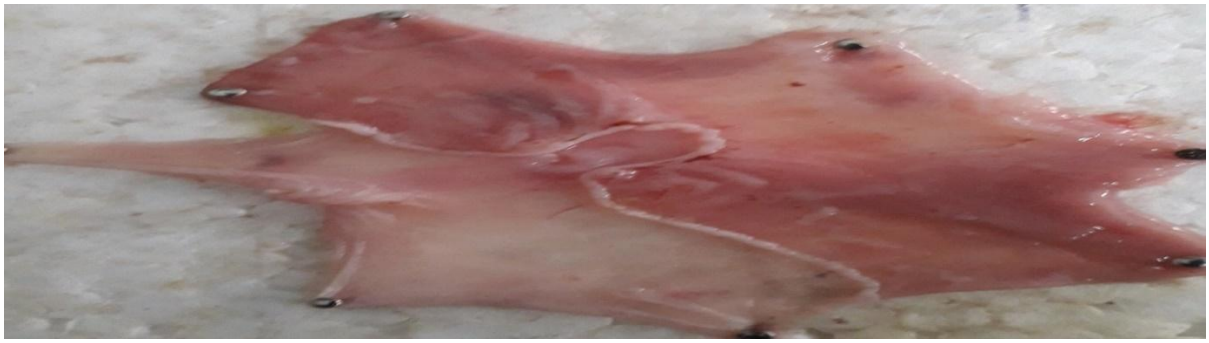

CI 100