Anti-ulcer activity of aqueous root extract of *Ensete ventricosum* (Welw.) Cheesman (Musaceae) in experimental rats

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This is to certify that the thesis prepared by Dejene Hailu entitled "Anti-ulcer activity of aqueous root extract of *Ensete ventricosum* (Welw.) Cheesman (Musaceae) in experimental models of gastric ulcer 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 concerning originality and quality.

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ABSTRACT

Anti-ulcer activity of aqueous root extract of *Ensete ventricosum* (Welw.) Cheesman (Musaceae) in experimental rats

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Peptic ulcer is a chronic disease of gastrointestinal system caused by an imbalance between damaging factors and protective factors. Even though many antiulcer drugs used to treat this disease are available, most of these drugs produced undesirable side effects, microbial resistance and danger of drug interactions during therapy. An ethnobotanical study showed that *Ensete ventricosum* is used for treatment of peptic ulcer but the efficacy and safety are not established. Thus, this study was aimed to evaluate the anti-ulcer activity of aqueous root extract of *Ensete ventricosum* (*E. ventricosum*) in experimental rats. The effect of the extract on gastric ulcer was evaluated against indomethacin, ethanol and pyloric ligation-induced ulcer models at doses of 100, 200 and 400mg/kg. Dose levels were selected based on outcome of acute toxicity study. Pantoprazole at a dose of 40mg/kg was used as a standard drug while distilled water was used as negative control. In indomethacin and ethanol-induced ulcer, pretreatment with aqueous root extract of *E. ventricosum* significantly reduced level of gastric mucosal ulceration and improved ulcer protection. Furthermore, histopathological findings of rats pretreated with aqueous root extract of *E. ventricosum* also revealed gastroprotective activity. In pyloric ligation-induced ulcer, administration of *E. ventricosum* at a dose of 200 and 400mg/kg significantly ($p<0.05$) reduced both ulcers number and severity scores and histopathological findings also revealed gastroprotective activity of the extract. The extract at a dose of 400mg/kg exerted best ulcer protection (98.53%) which was comparable to standard drug in pyloric ligation-induced ulcer as compared to other models studied. The extract at a dose of 200 and 400mg/kg also displayed antisecretory activity as revealed by significant ($p<0.05$, $p<0.001$) reduction in volume, free and total acidity of gastric juice with significant ($p<0.05$, $p<0.001$) increment in pH of the gastric juice in both ethanol and pyloric ligation-induced ulcer in dose dependent manner. In conclusion, the aqueous root extract of *E. ventricosum* showed significant antiulcer activity.

KEYWORDS: Anti-ulcer, *Ensete ventricosum*, Ethanol-induced, Indomethacin-induced, Pantoprazole, pyloric ligation-induced, Rats

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Contents

LIST OF ABBREVIATIONS& ACRONYMS

ANOVA	Analysis of Variance
cAMP	cyclic Adenosine Mono Phosphate
CAT	Catalase
CDC	Center for disease control
cNOS	Constitutive nitric oxide synthase
COX	Cyclo-oxygenase
DDC	Diethyldithiocarbamate
EGF	Epidermal growth factor
eNOS	Endothelial nitric oxide synthase
GSH	Glutathione
H. pylori	*Helicobacter pylori*
IL-1β	Interleukin-1β
IL-6	Interleukin -6
IR	ischemia-reperfusion
iNOS	Inducible nitric oxide synthase
NSAIDS	Non-steroidal anti-inflammatory drugs
NO	Nitric Oxide
OECD	Organization for Economic Co-operation and Development
PG	Prostaglandin
PGE_2	Prostaglandin E_2
PGI_2	Prostaglandin I_2
PUD	Peptic ulcer disease
MDA	Malondialdehyde
SNNPR	Southern Nations, Nationalities and Peoples Regional State
SOD	Superoxide Dismutase
SPSS	Statistical Package for Social Science
TNF-α	Tumor necrosis factor-α

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1. INTRODUCTION

1.1 BACKGROUND

Peptic ulcer disease (PUD) is a chronic disease of gastrointestinal system characterized as an erosion in a segment of the gastrointestinal mucosa that penetrates through the muscularis mucosae, into the submucosa or more profound (Manchala, 2019; Abdulla *et al.*, 2014). PUD is the most prevalent among the gastrointestinal tract diseases and most common chronic illnesses among working-age adults which affect around 5–10% of the world population with an incidence of 0.1–0.3% per year (Tarasconi *et al.*, 2020; Teshome *et al*., 2019). It affects 1-2 per 1000 people annually and with an overall mortality rate of 10 to 15% (Sverden *et al*., 2019; Rao *et al*., 2012). Peptic ulcer usually occurs in the stomach and duodenum parts of the gastrointestinal tract because they are bathed by acid-pepsin secretion (Rai *et al.*, 2015). In addition, it can also be found at the lower end of the esophagus or in Meckel's diverticulum (Zeeyauddin *et al*., 2011).

Peptic ulcers are broadly classified into two based on the location where the ulcer is found: gastric ulcer, formed in the lining of the stomach which is caused because of damage to the coating of the stomach, and duodenal ulcer develops in the lining of the duodenum which is associated with corrosive action of gastric secretions on the surface epithelium of the small intestine (Manchala, 2019). Gastric ulcer is among the most serious type of gastrointestinal diseases which can represent as a malignancy and is a very common global problem today. Duodenal ulcers are more common in adult males whereas gastric ulcers occur commonly at advanced age group with a peak incidence reported in individuals aged above 60 years and in individuals with lower socio-economic status (Zeeyauddin. *et al.*, 2011).

Even though the exact etiopathogenesis of peptic ulcer is still not completely understood, it is believed to be due to an imbalance between the defensive factors (mucus, bicarbonate and prostaglandins) and damaging factors (acid, pepsin, bile salts and *helicobacter pylori (H. pylori*)) (Beiranvand and Bahramikia, 2020; Abdulla *et al*., 2016). Factors that may contribute to the occurrence of peptic ulcer includes gastric cancer, infections (*H. pylori*), chronic alcohol intake, smoking, excessive stress, and chronic usage of non-steroidal anti-inflammatory drugs (Karimulla and Kumar, 2012). These agents have been implicated in the pathogenesis of ulcer especially gastric ulcer through enhancing the damaging factors by diminishing defensive factors which

includes enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth and diminished gastric blood flow (Raju *et al*., 2009).

In additions, tumor necrosis factor-α (TNF- α), reactive oxygen species (ROS), incidence of apoptosis and bile acids secretion are other factors responsible for progression of peptic ulcers (Yan *et al.*, 2015; Kaur *et al*., 2012; Karpagam *et al*., 2011).

Non-steroidal anti-inflammatory drugs (NSAIDS) are implicated in acute and chronic ulceration of the gastric mucosa and in the pathophysiology of gastric and duodenal ulcer. Gastric ulcer induced by NSAIDs is caused by their interference in prostaglandins synthesis through inhibiting the expression of the enzyme cyclo-oxygenase 1 (COX-1) in particular. By doing so, NSAIDs impair the mucosal barrier and expose gastric mucosa to corrosive action of acid and pepsin (Kim *et al.*, 2016; Kaur *et al*., 2012). Study conducted in 2019 has indicated that approximately 10% of peptic ulcers are caused due to chronic consumption of NSAIDs and are more strongly linked to gastric ulcers than duodenal ulcers (Sverden *et al*., 2019).

The other well-established factor in the pathogenesis of gastric ulcer is TNF-α which is a major pro-inflammatory cytokine responsible for initiating inflammatory process that results in formation of gastric ulcers (Selim *et al*., 2016; Song *et al*., 2004). In addition, TNF-α also activates the extrinsic apoptotic signaling pathway via death domain receptor and through initiation of caspase particularly caspase-3 which finally leads to gastric ulceration and damage (Katary and Salahuddin, 2017).

Evidences indicate that reactive oxygen species have also been implicated in the etiology and pathophysiology of gastrointestinal inflammation and gastric ulcers by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage (Singh *et al*., 2012). Oxygen derived free radicals that are derived from metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells are cytotoxic and promote tissue injury and contribute to damage of gastric mucosa (Tan *et al*., 2013). Hence, agents that minimize and repair free radical-induced damage can play an appreciable role in healing the ulcer. Agents with potent antioxidant actions have been reported to be effective in promoting the defensive mechanisms thereby enhancing cytoprotection and/or healing in the experimentally induced peptic ulcers (Onasanwo *et al*., 2010).

The induction of gastric ulcers by histamine administration has long been recognized and is mediated through stimulation of H_2 receptor which results in enhanced gastric acid secretion and

vasodilation. Agents that block histamine receptors such as H_2 receptor blocker exhibit better therapeutic efficacy against histamine-induced ulcer (Namdeo *et al*., 2010).

H. pylori is highly related with pathogenesis of peptic ulcer diseases and recurrence. *H. pylori* is a gram-negative microaerophilic, flagellated, spiral shaped bacterium which selectively found on the surface of gastric epithelial cells and gastric mucosa (Sanaii *et al.*, 2019). It damages the tissue of the stomach and the first lining of the intestine through weakening the protective mucous coating of the stomach and duodenum. It also secretes an enzyme urease, an enzyme that catalyzes urea to ammonia, which neutralize the gastric acid and create favorable environment for bacterial survival and replication (Bhattamisra *et al*., 2019). It has been shown that infection with *H. pylori* is responsible for causing approximately 90% of duodenal ulcers and 70% of gastric ulcers (Sverden *et al*., 2019).

Studies are reported that cigarette smoking is also considered to be one of an important risk factor for development, maintenance, and recurrence of peptic ulcer diseases. Smoking cause gastric mucosal ulceration through different ways; one of which is by inhibiting mucosal cell proliferation in human and animal mucosal cells due to reduction in epidermal growth factor (EGF) synthesis (Li *et al*., 2014). Cigarette smoke and its active ingredients can also induce cell apoptosis in the esophagus, gastric mucosa and in the inner layers of the small intestine which is mediated through reactive oxygen species (ROS) (Li *et al*., 2014). In addition, cigarette smoke and its active compounds, such as nicotine can increase gastric acid secretion that accompanied by a reduction in bicarbonate production. Ample evidences suggest that smoking can interfere with the gastric mucosal immune system and increases susceptibility to *H. pylori* infection. Furthermore, it also decreases the blood flow to the gastric mucosa that result in inhibition of mucus synthesis and finally leads to gastric damage (Li *et al*., 2014).

1.2 Treatment of peptic ulcer disease

Treatment of PUD is usually directed at identifying and eliminating the risk factors and the major goal of treatment is relieving ulcer pain, healing the ulcer, preventing ulcer recurrence, and reducing ulcer-related complications. Non pharmacological treatment of PUD includes avoidance of alcohol and caffeine, discontinue or reduce NSAIDs ingestion, quit cigarette smoking, reduce psychological stress and avoid foods and beverages that exacerbate ulcer symptoms. Prior to the discovery of *H. pylori*, for many years the standard practice was acid suppression treatment. Several anti-ulcer drugs such as anticholinergic drugs, histamine H_2-receptor antagonists, antacids,

prostaglandin analogues (misoprostol) and proton-pump inhibitors have been used for management and prevention of peptic ulcer disease. However, after the discovery of the *H. pylori* the management protocol of ulcer was changed (Dipiro *et al*., 2014).

H_2 receptor antagonists (H_2RAs):

H_2RAs (cimetidine, famotidine, nizatidine, and ranitidine) exhibit competitive inhibition of acid secretion by blocking H_2 receptors on the parietal cell. They are effective in suppressing nocturnal and postprandial acid production since they inhibit basal and meal-stimulated acid secretion. Acid secretion stimulated by histamine, gastrin and cholinergic agents is inhibited by H_2 receptor antagonists through two mechanisms: First, they block binding of histamine released from enterochromafin like (ECL) cells by gastrin or vagal stimulation to the H_2 receptor on parietal cell. Second, though direct stimulation of the parietal cell by gastrin or acetylcholine responsible for acid secretion, in the presence of H_2 receptor blocker there is diminished effect (Katzung, 2018).

Proton pump inhibitors (PPI):

PPIs (omeprazole, esomeprazole, lansoprazole, dexlansoprazole, rabeprazole, and pantoprazole) are among the most potent and widely used drugs for treatment of peptic ulcer disease. It acts by inhibiting the action of gastric H^+/K^+-ATPase (proton pump), final common pathway of acid secretion. Thus, they are effective in acid suppression from both fasting and meal-stimulated secretion. PPIs inhibit 90–98% of 24-hour acid secretion when given in standard and equivalent doses since they irreversible inactivate the proton pump (Katzung, 2018).

Antacids:

They were the cornerstone for management of peptic ulcer disease until the advent of H_2–receptor antagonists and PPIs. They are weak bases which neutralize gastric acid and lead to reduction of intragastric acidity. Approximately 45 mEq/h of hydrochloric acid is produced after each meal. Administration of a single dose of 156 mEq of antacid 1 hour after a meal can efficiently neutralizes postprandial gastric hydrochloric acid secretion for up to 2 hours (Katzung, 2018). They are also used for management of gastroesophageal reflux disease (GERD) since they increase tone of esophageal sphincter and decrease regurgitation of gastric contents (Gulia and Choudhary, 2011)

Prostaglandin analogues:

Misoprostol is a synthetic methyl analog of PGE_1 that has both antisecretory and cytoprotective properties. Antisecretory effects are dose dependent which produce an effect at a dose range of 50

to 200 mcg and its' antisecretory effect is due to its binding to a prostaglandin receptor on parietal cells and reducing histamine stimulated cAMP (cyclic adenosine mono phosphate) production. It produces cytoprotective effect through stimulating mucus and bicarbonate secretion and by enhancing gastric mucosal blood flow at doses of greater than 200 mcg. (Katzung, 2018; Gulia and Choudhary, 2011).

Anticholinergics:

Pirenzepine, a relatively specific muscarinic M_1-receptor antagonist suppresses gastric acid secretion and decrease gastric motility. It inhibits basal and stimulated gastric acid secretion by up to 40% and prolongs the gastric emptying time. Currently, it is rarely used because of its' relatively poor efficacy, unwanted side effects and risk of blood disorders (Gulia and Choudhary, 2011).

Ulcer protectives:

Sucralfate, a salt of sucrose complexed to sulfated aluminum hydroxide that act as cytoprotective agent. Even though the exact mechanism of action is unclear, it proposed that it enhance prostaglandin synthesis, stimulate mucous and bicarbonate secretion as well as it act as a physical barrier through binding of negatively charged sucrose sulfate to positively charged proteins in the base of ulcers or erosion that impede further tissue injury by acid and pepsin. (Katzung, 2018).

Ulcer healers:

Carbenoxolone sodium (CBS) is a synthetic derivative of glycyrrhizinic acid used orally for the treatment of gastric ulcers. It has cytoprotective effect by stimulating mucus secretion that prevent detrimental effect of acid on gastric mucosa and promotes ulcer healing. Rebamipide, an amino acid derivative of 2-(1H)-quinolinone that exert a cytoprotective effect through enhancing prostaglandins synthesis and scavenging reactive oxygen species. Hence it accelerates the process of ulcer healing (Goodman and Parker, 2008).

Anti *H. pylori* drugs:

Eradication of this infection is the major goal of treatment in patients with peptic ulcer disease and the ideal regimen should have an eradication rate of at least 80%. Antibiotics that have been most extensively studied and found to clinically effective against *H. pylori* are: amoxicillin, clarithromycin, tetracycline and metronidazole. However, the use of any single antibiotics is relatively ineffective and associated with a higher rate of antimicrobial resistance (Dipiro *et al*., 2014; Goodman and Parker, 2008)

The PPI-based triple therapy is the mainstay of treatment for eradicating *H. pylori* and consisting of a proton-pump inhibitor (PPI) and two antibiotics, such as clarithromycin plus amoxicillin or metronidazole given for 7−14 days. In areas where local clarithromycin resistance rate is more than 15%, PPI-based triple therapy regimens should not contain clarithromycin. PPI-clarithromycin-amoxicillin or PPI-clarithromycin-metronidazole regimens can be used in areas with low resistance to clarithromycin or when individual susceptibility to clarithromycin has been confirmed (Lanas and Chan, 2017). For penicillin-allergic patients' metronidazole should be substituted for amoxicillin in PPI-based triple therapy regimens (Dipiro *et al*., 2014).

Bismuth-based quadruple therapy (bismuth salicylate, metronidazole, tetracycline, and either a PPI or H_2RA) are a second-line drugs for eradication of *H. pylori* in case of treatment failure with PPI-based triple therapy containing PPI-clarithromycin–amoxicillin regimen or if there is local resistance to amoxicillin and metronidazole. Previously, H_2RA were used instead of PPI in bismuth-based quadruple therapy but currently PPI containing regimens are preferably used because it provides greater efficacy and permits a shorter treatment duration (7 days) when compared with the H_2RA-based regimens (10 to 14 days). However, a 10- to 14-days duration is considered more appropriate, as it generally provides higher eradication rates and reduces relapse. Substitution of bismuth subsalicylate with bismuth subcitrate potassium (biskalcitrate) provides comparable *H. pylori* eradication rates. Similarly, eradication rates are comparable when clarithromycin 250 to 500 mg four times a day is substituted for tetracycline but increases adverse effects (Dipiro *et al*., 2014).

Sequential therapy is a new form of eradication therapy, which consists of dual therapy with a PPI and amoxicillin for 5-days followed by a triple therapy with a PPI, clarithromycin, and tinidazole or metronidazole for an additional 5 days. Overall, it has achieved an eradication rates that are superior to 7-day and 10-day triple therapy regimens but not better than the eradication rate of 14-day triple therapy and bismuth-based quadruple therapy (Lanas and Chan, 2017; Dipiro *et al*., 2014).

Novel anti-ulcer agents

Ilaprazole: is a newly discovered PPI that is currently marketed in South Korea and China. It is a benzimidazole that acts by irreversibly inhibiting H^+/K^+ -ATPase (Hunt and Scarpignato, 2018). It has an extended plasma half-life ($t_{1/2}$ -3-6h) and its metabolism is not significantly affected by CYP2C19 as compared to the delayed release PPIs (Hunt and Scarpignato, 2018). However,

compared to delayed release PPIs, ilaprazole has no superior effect in GERD and or *H. pylori* eradication (Hunt and Scarpignato, 2018).

Durasec™ (AGN201904-Z): is the sodium salt of an acid stable prodrug of omeprazole and is currently in phase I clinical trial in Canada. Compared to delayed release PPIs, it has an extended plasma half-life ($t_{1/2}$ -4.5h) with improved and extended inhibition of basal acid secretion (Hunt and Scarpignato, 2018). This agent is therefore having a promising antisecretory activity in peptic ulcer patients.

Tenatoprazole (TU-199): is a long acting imidazopyridine derivative which is currently in phase II clinical trial in Canada and Europe. It has long half-life ($t_{1/2}$ – 9h) when compared to delayed release PPIs and exerted an extended effect on suppression of nocturnal acid secretion (Hunt and Scarpignato, 2018). This agent has also a promising antisecretory activity in peptic ulcer patients.

Azeloprazole (E-3710 or Z-215): is benzymidazole derivative currently at the end of phase II clinical trial in Japan. It is a potent and long-acting agent which produced a long-lasting inhibition of gastric acid secretion. In addition, it is not affected by CYP2C19 (Hunt and Scarpignato, 2018).

Anaprazole: is also benzymidazole derivative currently at phase III clinical trial in China. It is a long-acting PPI (Hunt and Scarpignato, 2018).

DLBS-2411 (Redacid®): is a natural PPI currently at phase III clinical trial in Indonesia. It acts as a competitive inhibitor H+/K+-ATPase and decreases H+/K+-ATPase messenger RNA expression in rat gastric parietal cells in a dose dependent manner (Hunt and Scarpignato, 2018).

1.3 Rationales of the study

Globally, peptic ulcer disease and its complications remain a major health hazard that cause significant morbidity, mortality and economic burden (Yismaw *et al*., 2020; Daniel *et al*, 2017; Gelayee *et al*., 2017). It affects a considerable number of people and estimated that nearly 10% of the world population may suffer from peptic ulcer throughout their life (Ercan *et al*., 2020; Sahoo *et al*., 2016). Studies have shown that in sub-Saharan Africa, from patients who underwent surgery for PUD, 86% suffered from duodenal ulcers while the rest 14% had gastric ulcers (Rickard, 2016).

Despite the decline in the incidence and rate of hospital admission for uncomplicated peptic ulcer disease (PUD) in developed countries in recent years, there was a striking rise in admission for complications of peptic ulcer which includes ulcer hemorrhage, bleeding and perforation among advanced age groups. This could be due to various factors such as increased incidence of the *H. pylori* infection, increased use of non-steroidal anti-inflammatory drugs (NSAIDs), chronic use alcohols and cigarettes smoking (Shah *et al*., 2016; Roy et.al., 2013).

A number of drugs including proton pump inhibitors, H_2 receptor antagonists, antacids and anticholinergics are available for the treatment of peptic ulcer but most of these drugs produce undesirable side effects. For instance, H_2-receptor antagonists (e.g., cimetidine) may cause gynecomastia in men and galactorrhea in women whereas the use of proton pump inhibitors can cause nausea, abdominal pain, constipation/diarrhea and also electrolyte imbalances. Abdominal cramps, uterine bleeding and abortion may be associated with the use of prostaglandin analogues. Moreover, use of antacids leads to stomach distention, belching, constipation and risk of ulcer perforation. Whereas other drugs like anticholinergics induce constipation, dry mouth, urinary retention, blurred vision, xerostomia and precipitation of glaucoma. (Yismaw *et al*., 2020; Kaur. *et al*., 2012). In addition, studies have shown that these drugs may associated with development of tolerance and even increased emergence of drug resistance on long term use (Kadir *et al*., 2011).

The other major problems in the treatment of gastroduodenal ulcer with conventional agents is that, despite a healing rate of 80–100% after 4–8 weeks of therapy with H_2-antagonists and proton pump inhibitors, the rate of ulcer recurrence is between 40 and 80% within a year after suspending the treatment (Kommu *et al*., 2013; de Andrade *et al*., 2007).

The introduction of a modern and potent antiulcer agents like proton-pump inhibitors to the classic anti-ulcer therapy had improved the treatment protocol of peptic ulcer disease; but clinical evaluation of these drugs has shown that there is still no complete cure for this disease. Hence, nowadays researchers are emphasizing to search for an ideal anti-ulcer drug and new alternatives which affords better protection, more effective and safe anti-ulcer agents.

Currently, medicinal plants are among the most attractive sources of new drugs available as therapeutic agents providing numerous evidences that show great potentials of medicinal plants used in the treatment of a large range of ailments, including gastrointestinal disorders (Schmeda-Hirschmann and Yesilada, 2005). In addition, as high as 80% of the world populations and of this about 75–80% people in developing countries use herbal medicines as first line of choice for the treatment of diseases including peptic ulcer because of the easy availability, cultural acceptability, affordability and lesser side effects. (Kumar *et al*., 2011; Gelayee *et al*., 2017). Several medicinal plants have been reported to possess anti-ulcer activities that enhance defensive mechanisms through increasing synthesis of prostaglandins, stimulating the secretion of mucus and bicarbonate, accompanied by inhibition of acid secretion and has been proven to be the major approaches for therapy of peptic ulcer disease (Meneses Oliveira *et al*., 2010). This study was undertaken to investigate anti-ulcer activity of *Ensete ventricosum* in experimentally induced gastric ulcer models and can help to check its traditional claimed use by the traditional practitioners.

1.4 Literature review

1.4.1 Medicinal plants with reported antiulcer activities

Medicinal plants have been traditionally used for the treatment of different diseases including gastrointestinal disorders for centuries. Different parts of the medicinal plants have been shown to possess antiulcer activities. As regards to leaves of medicinal plants, the ethanolic leaves extracts of *Swietenia mahagoni* (Ahmed *et al*., 2012), *Salvadora Indica* (Sahoo *et al*., 2016), *Sesbania grandiflora* (Bhoumik *et al*., 2016), *Cupressus sempervirens* (Koriem *et al*., 2015), *Ficus pumila* (Thamotharan *et al*., 2012) and *Ochna obtusata* (Karimulla and Kumar, 2012) in different ulcer models revealed that the extracts exhibited significant antiulcer activities.

The aqueous leaves extract of *Nauclea latifolia* (Balogun *et al*., 2013), *Spondias mombin* and *Ficus exasperata* (Sabiu *et al*., 2016), *Murraya Koenigii* (Patidar, 2011) and *Plantago lanceolata* (Engidawork *et al*., 2011) were reported to possesses significant anti-ulcer activities against different models of ulcer.

Methanolic leaves extract of *Osyris quadripartita* (Gelayee *et al*., 2017), *Bauhinia purpurea* (Zackaria *et al*., 2012) and *Abutilon indicum* (Dashputre and Naikwade, 2011) exhibited significant antisecretory and cytoprotective property in pylorus ligated and ethanol-induced ulcers in rats.

Aqueous, chloroform and ethanol extract of the leaves of *Lawsonia Inermis* employing the pylorus ligation and aspirin-induced models possess significant gastroprotective properties (Goswami *et. al*., 2011). The petroleum ether, alcohol and water extracts of *Hibiscus rosa* possessed anti-ulcer activity in pyloric ligation induced gastric ulcer model in albino rats (Srivastava *et al*., 2013). The study conducted in Eastern Himalaya on the leaves of *Amaranthus spinosus* in ethanol and cysteamine-induced gastric ulcer showed that the extract exerted anti-peptic ulcer activity (Mitra *et al*., 2013).

An experimental study conducted on the bark extracts of *Boswellia serrata* (Zeeyauddin *et al*., 2011), *Garuga pinnata* (Sachan *et al*., 2014), *Scutia buxifolia* (Boligon *et al*., 2014) and *Balanites aegyptiaca* (Kommu *et al*., 2013) revealed that the extracts exhibited significant antiulcer activities against different gastric ulcer models.

With regards to root of medicinal plants, ethanolic extracts of *Buchanania lanzan* (Pareta *et al*., 2010), hydroalcoholic crude extract and solvent fractions of *Rumex nepalensis* (Zewdu and Aregaw, 2020), *Polygonum cuspidatum* (Kim *et al*., 2020) and Methanolic extract and solvent

fractions of the root of *Croton macrostachyus* (Mekonnen *et al*., 2020) possesses significant anti-ulcer activities against different experimentally induced gastric ulcer models.

An experimental study carried out to evaluate the anti-ulcer activities of ethanolic extract of fruits of *Terminalia belerica* (Choudhary *et al*., 2012), methanolic extract of the fruits of *Piper cubeba* (Parvez *et al*., 2010) and ethanolic extract of *Cordia Myxa* fruit extract (Inas *et al*., 2011) revealed potential antisecretory and cytoprotective activities against different ulcer models. As far as seeds extract of plant materials is concerned, aqueous extract of *Nigella sativa* in aspirin-induced ulcer models in albino rats (Sengupta *et al.,* 2013), ethanolic extract of *Myristica fragrans* seeds on ethanol-induced ulcer in albino rats (Sattar *et al*., 2019) and methanolic extract of *Cordia africana* seeds in pyloric ligated rats (Yismaw *et al*., 2020) possess prominent antiulcer activities.

The study conducted to investigate antiulcer activity of aqueous extract of fermented unripe *Musa paradisiaca* fruits using acetic acid, aspirin, ethanol, indomethacin and pyloric ligation-induced ulcer models has revealed a good anti-ulcer activity (Ugbogu *et al*., 2017). Furthermore, the study done to evaluate the anti-ulcer activity of aqueous extract of *Musa sapientum* against indomethacin-induced gastric erosions in animal models showed that the extract possesses anti-ulcer activity (Karpagam *et al*., 2011).

1.4.2 Overview of the experimental plant

Ensete ventricosum is an important multipurpose indigenous crop categorized under a perennial, herbaceous, monocarpic and monocotyledonous plant which belongs to the family Musaceae and genus *Ensete* (Agren and Gibbson, 1968; Belachew and Gebre-Mariam, 2009). It is commonly known as false banana, the Ethiopian banana or the Abyssinian banana because it is related to and has physical resemblance with the banana plant (Berhanu *et al*., 2018; Tesfaye and Girma, 2017; Tesfaye *et al*., 2016,). Botanically, it is named as *Ensete ventricosum* (Welw.) Cheesman which is locally called Enset (Amharic) and Warqee (Afan Oromo). *Ensete ventricosum* is the only species of the genus *Ensete* that has been domesticated, most cultivated and consumed as a crop (Yemataw *et al*., 2017). It is an endemic multipurpose crop in Ethiopia and has remarkable significance in our day-today-life through ensuring year-round food and feed security, cloth, house, bed, cattle-feed and plate. It is a rich source of starch and utilized in different food preparations which are abundant in starch namely, Kocho, Bulla, Amicho and Workay (Tesfaye and Girma, 2017; Tesfaye *et al*., 2016). Approximately 20 million people in the south and south western Ethiopia consume *Ensete* as a staple/co-staple food (Nuraga *et al*., 2019). Additionally, the subsequent yield of

Ensete, the flour when mixed with *Eragrostis tef* is used to make 'Injera'' (traditional Ethiopian bread) (Singh *et al*., 2019). It is also widely used to prepare food for celebration of festivity during wedding, holydays, and birthday in the Shekicho community (Garedew *et al*., 2017).

Even though, it is widely cultivated in south and southwestern Ethiopia, it is also cultivated in other parts of the country including Lake Tana, the Simien Mountains, western Bale, south and east Shewa, Jimma, Illubabor, Welega, and as far north as Adigrat (Yemataw *et al*., 2018; Tesfaye and Girma, 2017). *Ensete* agriculture contributes to soil fertility and environmental sustainability as any perennial crop; avoiding erosion and keeping nutrients and moisture (Abdella, 2016).

1.4.3 Medicinal uses of *Ensete*

Ensete is widely utilized in Ethiopia for having great medicinal value for healing of bone fracture, joint displacement and swelling with pus (Tesfaye *et al*., 2016; Tsehaye and Kebebew, 2006). The boiled corm Amicho and starchy powder bulla are eaten with milk for this purpose. The uses of *ensete* in traditional treatment of bone related illness may be due to their phytoconstituents that are responsible for wound healing or their electrolyte contents like calcium and phosphorous which are known to have biological importance (Singh *et al*., 2019). The corm Amicho of *ensete* is used to stimulate the placental discharge in both cattle and humans (Maryo *et al*., 2018; Tesfaye *et al*., 2016; Tsehaye and Kebebew, 2006). In Shekicho people, shuri varieties of e*nsete* is utilized for abortion purpose as home medication (Garedew *et al*., 2017). In addition, it has been proposed that taking *kocho* or *Bula* after taking other traditional medicine minimizes the side effect and are widely practiced by shekicho people in sheka zone (Garedew *et al*., 2017). *Ensete* is endowed with activities against diseases caused by pathogens including several bacteria, nematodes, and viruses (Yemataw *et al*., 2018; Tesfaye and Girma, 2017; Tesfaye *et al*., 2016). The watery liquid squeezed from the pseudostem of Hargama and Moche is used to heal fungal disease called *Tinea corporis* by washing the infected skin with boiled varieties (Maryo *et al.,* 2018). The active constituents isolated from seeds of *Ensete superbum* particularly nonsteroidal phytosterol (4-hydroxy-3-methyl-hex-5-enyl), is used as food additive to reduce cholesterol (Tesfaye *et al.*, 2016). It has been reported that *Ensete* species are also used to treat a different type of diseases such as cancer, toothache and stomach ache (Nuraga *et al.*, 2020). The study on genetic diversity of *Ensete ventricosum* landraces has been shown that *Ensete* varieties are commonly used for treatment of liver disease, cough, skin itching and back injury (Nuraga *et al.*, 2020). It has been reported that another species of *Ensete, Ensete superbum*, is also used for management of different types of

conditions such as: - inflammation, infertility and kidney stone (Singh *et al*., 2019). Moreover, seeds and pseudostems of *Ensete superbum* are shown to have antiurolithiatic activities whereas seeds and whole plant of this species are reported to possess antidiabetic effect (Singh *et al.*, 2019). An ethnomedicinal study done on *Ensete superbum* reported that seeds and whole plant of this species are used for treatment of viral infection like dog bites (Singh *et al*., 2019). Starch, a major constituent of *Ensete*, have a biological active component that have been reported to possess pharmaceutical application as natural binder, disintegrator, super-disintegrants and gelling agent (Tesfaye and Girma, 2017; Tesfaye *et al*., 2016, Gabriel *et al*., 2013). An ethnopharmacological survey done on plant materials revealed that *E. ventricosum* leaves mixed with other plant leaves is used to treat diarrhea (Balemba *et al*., 2013). Another ethnobotanical study done on medicinal plants in West Shewa Zone of Oromia Region showed that *Ensete ventricosum* (Welw.) Cheesman is used for treatment of peptic ulcer disease (Duressa, 2016).

Fig. 1. Photograph of *Ensete ventricosum*

1.4.4 Phytoconstituents of plants of the genus *Ensete*

The study done on preliminary phytochemical analysis of aqueous root extract of *Ensete ventricosum* has revealed the presence of secondary metabolites such as tannins, saponins, glycosides, flavonoids, phenols, coumarins and free sugars (Wainaina, 2019). On the other hand,

it has been reported that preliminary phytochemical analysis of methanolic root extract of *Ensete ventricosum* revealed the presence alkaloids, glycosides, saponins, steroids, flavonoids, terpenoids, phenols, coumarins and free sugars (Wainaina, 2019). It has been reported that various phytoconstituents have been reported from *Ensete superbum* including alkaloids, saponins, tannins, phenols, flavonoids, anthraquinone glycosides, steroids and amino acids (Mishal and Shirsat, 2020)

1.4.5 Experimental peptic ulcer models

Experimental induction of peptic ulcer in vivo can be generated by pharmacological, physiological or surgical manipulations in several animal species. However, most in-vivo experimental peptic ulcer models are carried out in rodents. There are several models used for testing or evaluating anti-ulcer activity of drugs/agents, and these are described below.

1.4.5.1 Water-immersion stress or cold-water-restraint or cold-restraint stress induced ulcer

Water-immersion stress or cold-water-restraint or cold-restraint stress-induced ulcer model is widely used as experimental model for generation of acute gastric ulcer (Samanta *et al*., 2018). Of various factors which contribute to gastric ulcers, psychogenic factors like stress, play a very important role in the pathogenesis and formation of gastric ulcers in humans (Mishra *et al*., 2019). Stress-induced gastric ulcer is an acute gastric mucosal lesion mainly characterized by inflammatory erosion and gastrointestinal bleeding (Sun *et al*., 2020). Multiple mechanisms contribute to stress-induced ulcers; the most important of which appear to be due to the release of histamine, an increase in acid secretion, decreased biosynthesis of prostaglandin and poor flow of gastric blood (Shah *et al*., 2016). Stress also causes both sympathetic and parasympathetic stimulation. Sympathetic stimulation result in direct arteriolar vasoconstriction whereas parasympathetic simulation result in an increase in gastrointestinal motility and muscular contraction (Sun *et al*., 2020; Goel *et al*., 2004). In addition, it also facilitates generation of oxygen-derived free radicals by endothelial cells and polymorph nuclear neutrophils. Free radicals cause membrane lipid peroxidation by inducing ischemia and vascular endothelial cell damage, impaired ion transport and membrane integrity and finally loss of cellular functions (Sandrine *et al*., 201; Goel *et al.,* 2004). Endogenous antioxidants (superoxide dismutase, glutathione and catalase) are effective in preventing stress-induced ulceration of the gastric mucosa. Cooling of experimental animals in water during the restraint period facilitate formation gastric ulcer (Mishra *et al*., 2019).

Merits of this model are easy, effective and short time duration procedure to induce gastric ulceration in rats (Samanta *et al*., 2018). It is suitable for analyzing the antiulcer activity of the drug on the basis of physiology since this model mimic the disease condition in humans (Mishra *et al*., 2019). In addition, gastric ulcers induced by this model in rats or mice resemble human peptic ulcer, both grossly and histopathologically (Shah *et al*., 2016). The demerit of this model is that it is specific for the evaluation of cytoprotective activities of plant extract (Mishra *et al*., 2019).

1.4.5.2 NSAIDs- (indomethacin, aspirin, and ibuprofen) induced gastric ulcers

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, aspirin, and ibuprofen that are extensively used as analgesic, anti-inflammatory and antipyretic purpose are known to induce ulcerative gastric damage (Katary and Salahuddin, 2017). There are multiple mechanisms by which NSAIDs induce gastric ulcers. In cyclooxygenase pathway, NSAIDs suppress prostaglandin (PG) synthesis through inhibition of cyclooxygenases (COXs). This result in decreased bicarbonate and mucus secretion which further lead ulcer formation, epithelia damage and reduce angiogenesis (Kim *et al.*, 2016). All NSAIDs, except aspirin, when given at ulcerogenic doses causes increased gastric motility. This hypermotility causes microvascular disturbances and neutrophil-endothelial interaction leading to the development of gastric lesions (Takeuchi, 2012).

Increased production of nitric oxide (NO) due to the overexpression of inducible nitric oxide synthase (iNOS) is one of the mechanisms by which NSAIDs damages the gastric mucosa. NO is a known mediator of gastrointestinal mucosal defense but it also causes gastric mucosal damage (Katary and Salahuddin, 2017; Watanabe *et al*., 2011). It has been shown that different isoforms of nitric oxide synthase produce different concentrations of NO and produce different, often opposite effects in the same tissue. Indeed, low amounts of NO produced by mucosal and endothelial NOS (eNOS) isoforms protects the integrity of epithelial tissue, increase mucus production and accelerate ulcer healing. But high quantity of NO produced by iNOS has detrimental effect on gastric mucosa (Katary and Salahuddin, 2017; Watanabe *et al*., 2011).

Studies have suggested that reactive oxygen species (ROS) play a significant role in the pathogenesis of NSAIDs-induced gastric ulcers (Suleyman *et al*., 2020). ROS cause marked depletion of the innate antioxidants like superoxide dismutase (SOD), catalase and glutathione (GSH) and in turn increase in tissue malondialdehyde (MDA). MDA can damage the membrane proteins by inactivating the membrane receptors and membrane-related enzymes through the

cross-linking and polymerization of the components of the membrane (Suleyman *et al*., 2020; Datchanamurty *et al*., 2019). NSAIDs, particularly indomethacin causes the release of cytochrome C from mitochondrial inter membranous space into cytosol and the release of ROS through inhibition of the mitochondrial oxidative phosphorylation. This result in leakage of Ca2+ out of mitochondria, cellular osmotic imbalance and lipid peroxidation which finally leads to increased permeability and subsequent gastric mucosal damages (Katary and Salahuddin, 2017).

NSAIDs –induced apoptosis is another mechanism that is involved in the pathogenesis of gastric ulcer. NSAIDs induces apoptosis through up regulation of the intrinsic pathway caspase 9 and activation of caspase 3 in turn leads to cell death (Katary and Salahuddin, 2017).

Merits of this model is that it is a suitable method for detection of the effect of the antiulcer drug on gastric blood flow. It is also an effective method for the detection of the effect of COX inhibitors on leukocyte adherence. The demerits of this model is that it is required to inhibit both COX-1 and COX-2 for NSAIDs-induced gastric ulcer in rats because in most of the cases when given individually neither a COX-1 nor a COX-2 inhibitors cause macroscopically or histologically detectable gastric damage (Mishra *et al*., 2019).

1.4.5.3 Ethanol-induced gastric ulcers

Ethanol-induced gastric ulcer is basic experimental model used to evaluate antipeptic ulcer activity of various drugs and natural products especially in animal model studies since it can easily penetrate the gastric mucosa and causes gastric ulcer (Kim *et al*., 2015). The pathogenesis of ethanol-induced lesion of the gastric mucosa occurs through different mechanisms. Ethanol causes necrotic lesions of the gastric mucosa by disrupting the mucus-bicarbonate barrier. This results in cell rupture in the wall of blood vessels which in turn reduces defensive factors like bicarbonate, mucus and mucosa circulation (Abdulla *et al*., 2012). Ethanol produces a marked contraction of the circular muscles of rat fundic strip and disrupts the gastric mucosal barrier which causes a profound micro-vascular change that leads to necrosis and ulceration (Abdulla *et al*., 2012).

Studies have shown that inflammation has been well linked to the pathological process of ethanol-induced gastric ulcer (Wang *et al*., 2020). Ethanol consumption induces the inflammatory response that initiates the innate immune system leading to the release vast amounts of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α (Wang *et al*., 2020; Yan *et al*., 2015). Elevated levels of these pro-inflammatory cytokines promote the accumulation of neutrophils, lymphocytes and monocytes at the inflammation site. These initiates different oxidative bursts, toxic metabolites

and lysosomal enzymes that leads to the gastric mucosal inflammation, destruction of the mucosal barrier, mucosal vascular congestion and injury (Wang *et al*., 2020; Yan *et al*., 2015).

The pathogenesis of ethanol-induced mucosal damage in the stomach also results from the involvement of reactive oxygen species such as superoxide anions, hydrogen peroxide and hydroxyl radicals. These oxygen-derived free radicals play a vital role in the formation of lipid peroxides accompanied by impairment of antioxidative enzyme activity of cells that result in gastric oxidative stress, gastric hemorrhage and ulcer formation (Abdulla *et al*., 2014).

It has been reported that neutrophil infiltration into the gastric mucosa plays a vital role in the pathogenesis ethanol-induced gastric ulcer. TNF-α, a major pro-inflammatory cytokine is responsible for stimulating neutrophil infiltration in gastric inflamed areas (Elsayed *et al*., 2019). Elevation of the gastric myeloperoxidase (MPO) activity released from neutrophils is used to estimate the accumulation of neutrophil infiltration into the gastric mucosal tissues. (Elsayed *et al*., 2019; Li *et al*., 2013). Excessive production of MPO causes cell membrane damage by lipid peroxidation which result in gastric mucosal damage (Abdulla *et al*., 2012). Nitric oxide, derived from constitutive nitric oxide synthase is responsible for the maintenance of normal gastric mucosal integrity and healing of gastric ulcer that may be due to its ability to reduce neutrophil infiltration (Elsayed *et al*., 2019).

Evidences have revealed that ethanol-triggered gastric injury also caused due to enhancement of the apoptotic pathway. Ethanol ingestion increases protein expression of the proapoptotic Bax which in turn causes release of cytochrome-c followed by activation of caspase cascade and finally leads to apoptotic gastric mucosal cells death (Lebda *et al*., 2018).

Furthermore, studies have shown that ethanol consumption is associated with increased protein expression of iNOS and the decline of the gene expression of eNOS in gastric tissue. Increased concentration of NO generated from iNOS have been associated with severe gastric inflammation in ulcerative mucosal tissues and it had a critical role in the enhancement of gastric ulcer (Lebda *et al*., 2018).

Merits of ethanol-induced ulcer is that it is the most accepted and a preferred model used to evaluate gastro-protective activity of plant materials in experimental animals (Sidahmed *et al.* 2019). It is reproducible method to produce gastric lesions in experimental animals and choice of model for evaluation antiulcer activity (Mishra *et al.*, 2019). With regards to demerit of this model,

it is a risk factor for developing gastric ulcers as it penetrates the gastric mucosa and cause damage to the cell membrane Mishra *et al.*, 2019).

1.4.5.4 Acetic acid-induced gastric ulcers

Acetic acid-induced ulcer model has been widely used to conduct pharmacological and pathophysiological studies of chronic gastric ulcer (Wang *et al*., 2020). In this model, not only rats, but also other animals like cats, dogs, guinea pigs, Mongolian gerbils, miniature pigs and monkeys are used as experimental animals for induction of ulcer (Mishra *et al*., 2019). Induction of chronic gastric ulcer can be established by injection or topical application of acetic acid solution into the stomach (Bi *et al*., 2014). The pathophysiology of acetic acid-induced gastric ulcers are multifactorial. Chronic ulcers induced by acetic acid are mainly due to the release of histamine, which increases the capillary permeability and back diffusion of HCl that result in increased volume of gastric juice. These increment in the volume of gastric juice causes mucosal injury that was confined to the glandular stomach with subsequent pyloric obstruction and mucosal necrosis (Arun *et al*., 2010).

The application of acetic acid to the gastric mucosa induces a state of acute stress that resulted in chronic oxidative stress. Gastric mucosal oxidative stress increases lipid peroxidation with concomitant decrease in SOD activity and GSH levels which in turn leads to depletion of gastric wall mucus content and development of gastric ulcers (Xu *et al*., 2013).

Merits of this model are better resembling to human peptic ulcer disease in terms of pathological features, location, chronicity, severity and the healing process and servers as the most reliable model to study ulcer healing process (Viana *et al*., 2019). It is a simple procedure, incidence of ulcer is 100% and gives the rapid result of ulcer severity (Mishra *et al*., 2019). This model also used for evaluation of antisecretory and cytoprotective effects of various drugs and natural products (Mishra *et al*., 2019). Demerits associated with this model is that it produces unfavorable result on other organs since submucosal injection produced ulcers penetrating entire gastric wall & adherence of ulcer based on adjacent organs (Mishra *et al*., 2019).

1.4.5.5 Histamine-induced gastric ulcers

Histamine, is one of the major neurotransmitters responsible for the regulation of stomach acidity, influencing the permeability of blood vessels, contraction of muscles and cerebral function. Skin, lungs and stomach are organs with highest concentration of histamine and lower amounts is found in the brain and heart (Fulga *et al*., 2019). Induction of gastric ulcer by histamine is mediated

through both enhanced gastric acid secretion and vasospastic effects of histamine (Kaithwas and Majumdar, 2010). Induction of gastric ulcer by histamine not only due to secretion gastric acid but also due to it causes gastric mucosa abnormal motility, reduction in mucus production as well as microcirculation (Fulga *et al*., 2019).

Merits of this model is that it is a suitable method to evaluate antiulcer activity because it produces 100% gastric ulceration. In addition, it is used for the determination of the antisecretory effect of drugs or medicinal plants in case of excessive gastric acid secretion. Demerit of this model is it needs long duration of administration histamine for induction of ulcer.

1.4.5.6 Reserpine-induced gastric ulcers

Reserpine is one of the most widely used drugs for inducing gastric mucosal damage. Reserpine cause lesions of the gastric mucosa through various mechanisms. It causes lesions of the gastric mucosa by reducing sympathetic activity with simultaneous increase in the cholinergic tone which leads to excessive acid secretion and increase in digestive motility (Fulga *et al*., 2019; Ma *et al*., 2010). Reserpine-induced gastric ulcers are also mediated by histamine release due to degranulation of the gastric mast cells which in turn causes gastric secretion and mucosal defects (Kaithwas and Majumdar, 2010).

Studies have shown that reserpine is said to elucidate ulcers through generating oxygen derived free radicals such as superoxide and hydroxyl radicals. Accumulation of oxygen free radicals accompanied by depletion in anti-oxidant enzymes levels in the body results in evident damage to the gastric mucosal cells which finally leads to peptic ulcers (Nwafor *et al*., 2011).

Merit associated with this model is that it can be suitably used for studying the acute as well as chronic ulcers. With regards to demerits, it is dose-dependent and acid dependent.

1.4.5.7 Serotonin-induced gastric ulcers

Serotonin is a monoamine neurotransmitter derived from an essential amino acid, tryptophan. It is synthesized both in the enterochromafin cells of the gastrointestinal mucosa and in serotonergic neurons of the central nervous system. As an enteric neurotransmitter, serotonin act on mesenteric vascular smooth muscle to cause vasoconstriction that reduces blood flow to gastrointestinal, leading to lesions of the gastric mucosa (Fulga *et al*., 2019). Gastric ulcers induced by serotonin is also due to a disturbance of gastric mucosal microcirculation (Kaithwas and Majumdar, 2010).

This model is used to study the etiology of gastric ulcers and as a tool in the search for new antiulcer drugs. The demerit of this model is that extent of gastric ulceration induced by is model is time dependent.

1.4.5.8 Pylorus-ligation-induced peptic ulcers

Pylorus ligation-induced ulcer was developed by Shay *et al*. in 1945 and is one of the easiest methods for the production of gastric ulceration in the rat (Fulga *et al*., 2019). Even though it is the oldest animal model for induction of gastric ulcer, it is still the most common method for the antiulcer drug development and to study the effect of drugs on gastric acid secretion and mucus secretion (Mishra *et al*., 2019; Bhoumik *et al*., 2016). In surgically model, pyloric ligation-induced ulcer, gastric mucosal lesions are particularly mediated due to accumulation of gastric acid and pepsin in the stomach (Fulga *et al*., 2019). Accumulation and increases the volume of the gastric fluid in the stomach occurs due to the ligation of pyloric end of the stomach (Mishra *et al*., 2019; Bhoumik *et al*., 2016). The resulting gastric mucosal lesions occurs due to self-digestion of the mucosa that leads to the breakdown of gastric mucosal barrier and development of ulcer in the stomach (Mishra *et al*., 2019, Bhoumik *et al*., 2016).

It has been reported that generation of free radicals plays an important role in the pathogenesis of pyloric ligation-induced ulcers. Generation of free radicals with concomitant depletion of antioxidant status, namely decreased SOD activity and GSH levels, increase acidity and peptic activity in gastric juice leads to damage of the gastric mucosa (Zaghlool *et al*., 2019).

Merits associated with this model is that it is the easiest and most accepted method for evaluation of antiulcer activity of the drug molecule. It is useful to evaluate the anti-secretory activity of medicinal plants or drugs. In addition, this model is also used for determination of the cytoprotective effect of drugs which increases the secretion of mucus in the stomach. Demerit of this model is that it is difficult to get expected result since ulcers are localized in the antrum of the stomach.

1.4.5.9 Diethyldithiocarbamate- (DDC)-induced peptic ulcers

Diethyldithiocarbamate is a class of dithiocarbamate anions known to be a copper-chelating agent (Yildirim *et al*., 2013). The pathogenesis of DDC-induced peptic ulcers is through suppression of gastric mucosal copper–zinc superoxide dismutase activity and decreased gastric mucosal blood flow (Yildirim *et al*., 2013).

Studies have revealed that diethyldithiocarbamate-induced ulcers are also mediated by generation of reactive oxygen species. These free radicals induced gastric mucosal lesions is due to decreasing SOD levels and increasing lipid peroxide levels in the gastric mucosa (Yildirim *et al*., 2013). The peculiar feature of ulcers formed in this model is that ulcers are mostly induced at the level of the anthral region of the stomach (Fulga *et al*., 2019). The particularity of this model is that acute glandular lesions are induced by subcutaneous injection of diethyldithiocarbamate followed by the oral administration of hydrochloric acid (Fulga *et al*., 2019).

Merit of this method is that it is used to study the anti-oxidative activity and the cytoprotective effect of drugs. As regards to demerits, this method is not useful for evaluating antisecretory effect of medicinal plants/ drugs.

1.4.5.10 Methylene blue-induced ulcers

Methylene blue is a synthetic drug that has been known as an ulcerogenic agent in induction of duodenal and gastric ulcers in rats. It is known to uncouple ATPase and consequently activating H^+/K^+- ATP-ase (Shah *et al*., 2006). Methylene blue causes lesions of the gastric mucosa by increasing H^+/K^+- ATPase activity that increase hydrochloric acid secretion (Fulga *et al*., 2019). In addition, methylene blue has an affinity for acetylcholine at muscarinic receptors and inhibits cholinesterase activity which in turn increase in H^+/K^+ ATPase activity and finally leads to ulcers formation (Shah *et al*., 2006). It also inhibits constitutive nitric oxide synthase that causes a depletion in production of nitric oxide in turn results in reduced blood flow to the gastric mucosa and finally leads to formation of peptic ulcers (Fulga *et al*., 2019).

Moreover, reduction of blood flow to the gastric mucosa cause increased oxidative stress revealed by reduced in glutathione levels and catalase activity and subsequently produces gastric mucosal ulcer (Fulga *et al*., 2019; Shah *et al*., 2006,).

Methylene blue-induced ulcer is used as a pharmacological tool to screen the mechanisms of anti-ulcerous agents. It is also used to assess anticholinergic activity and proton pump inhibitory activity of drugs. The demerit of this model is that it will stain body fluids and tissues that may interfere with gross examination of ulcers

1.4.5.11 Ischemia-reperfusion- (I-R-) induced gastric ulcers

Ischemia-reperfusion-induced gastric ulcers is an important model for studying acute gastric mucosal lesions because gastrointestinal mucosa is very sensitive to ischemia. Ischemia-reperfusion- induced gastric ulcer in humans is a surgical induced gastric ulcer model that involves

lesions of the muscle mucosa as a consequence of compression of the celiac artery mostly by the median arcuate ligament (Fulga *et al*., 2019; Olaleye *et al*., 2016).

Experimental evidences suggest that generation of reactive oxygen species (ROS) due to reperfusion of gastro intestine following ischemia play a major role in the development of erosion and ulceration in the gastric mucosa (Mard *et al*., 2016). Xanthine-oxidase (XO) system is a biochemical mechanism which allow these free radicals to be produced. Xanthine-oxidase is the enzyme responsible for the conversion of xanthine and hypoxanthine to uric acid with the release of superoxide radicals and hydrogen peroxide (Lastra *et al.,* 2001).

It has been shown that production of proinflammatory cytokines including TNF-a, IL-1b and IL-6 are also responsible for the pathogenesis of ischemia-reperfusion-induced gastric ulcers (Noda *et al*., 2018). Prolonged upregulation of TNF-a, IL-1b and IL-6 due to ischemia causes inflammation of the gastric mucosa which results in formation of peptic ulcer (Noda *et al*., 2018).

Apoptosis is also another mechanism by which ischemia-reperfusion (IR)-induces gastric mucosal injury. IR injury in the gastric mucosa causes a rise in tissue total calcium level which in turn causes opening of the mitochondrial membrane permeability transition pore. This results in modulation of mitochondrial function (i.e., calcium overload, phospholipase activation and inhibition of respiratory chain function) leading to mitochondrial membrane dysfunction, ATP depletion, DNA fragmentation and apoptosis and gastric mucosal damage (Muthuraman *et al*., 2011).

Moreover, induction of gastric ulcer by ischemia reperfusion (IR) occurs with a decrease in constitutive NOS (cNOS) activity and a drastic increase in inducible NOS (iNOS) activity (Gou *et al.,* 2011). The increased production of NO by iNOS causes an impaired gastric mucus synthesis which leads to gastric mucosal lesions.

Merits of this model is that it can be used to study the pathophysiological mechanisms in the formation of peptic ulcer and to investigate antiulcer activity of new substances.

1.4.5.12 Cysteamine-induced duodenal ulcers

A duodenal ulcer induced by cysteamine was first introduced by Seley and Szabo in 1973 (Mishra *et al*., 2019). For induction of duodenal ulcer using cysteamine, a mouse is very convenient experimental animal because of smaller size and it requires few treatments for rapid production of ulcer which are similar to human disease. Even though the exact mechanism is not been fully elucidated, evidences indicated that it causes duodenal ulcer through stimulating gastric acid

secretion and inhibiting mucus secretion from Brunner's glands in the proximal duodenum (Krishnamurthy *et al*., 2018).

It has been shown that cysteamine markedly elevates serum gastrin levels by reducing somatostatin bioavailability and cause a significant decrease in the neutralization of acid in the proximal duodenum. This leads an increase in gastric acid secretion and accumulation. It also causes inhibition of gastric emptying and motility that finally leads to formation of duodenal ulcers (Krishnamurthy *et al*., 2018).

Oxidative stress and generation of ROS are also an important factor underlying the pathophysiologic mechanisms of cysteamine-induced duodenal ulcer in animals (Rezvanjoo *et al*., 2010). Enhanced free-radical levels like hydrogen peroxide, hydroxyl radical and an impaired in-cell antioxidants pool such as superoxide dismutase (SOD) causes cellular and tissue injury which leads to the development of duodenal ulcers

Merits of this model is that it resembles human ulcers in terms of location, histopathology and some aspects of pathophysiology**.** It is an easily reproducible and an acceptable method for evaluation of antiulcer. Demerit associated with this model is that it produces unfavorable result on other organs since ulcers are located on the anterior wall, the walls frequently get perforated resulting in peritonitis or penetrate into the liver and pancreas.

1.4.5.13 Indomethacin-histamine-induced duodenal ulcers

Indomethacin-histamine-induced ulcers is another model for inducing duodenal ulcers and was first developed by Takeuchi in 1986 (Grover *et al*., 2001). Induction of duodenal lesions by indomethacin plus histamine is due to both an increase in gastric acid secretion and an impairment of acid induced duodenal bicarbonate secretion. It has been reported that combined treatment of indomethacin plus histamine induce one or two round lesions in the proximal duodenum and a few lesions in the corpus and antrum of the stomach (Adinortey *et al*., 2013). In addition, this model is also used for rapid ulcer formation. It is useful for the evaluation cytoprotective and anti-secretory activity of drugs since increases acid secretion is mediated by histamine and inhibition the mucosal defense is due to indomethacin administration (Grover *et al*., 2001; Mishra *et al*., 2019).

Merits of this model is that it is a simple procedure with 100% gastric ulcer formation and cause no mortality. Demerit related to this model is that it produces ulcers penetrating entire gastric wall & adherence of ulcer base to liver when injected.

1.4.5.14 ferrous iron-ascorbic acid-induced gastric ulcers

This model is another method for inducing gastric ulcers and induction of gastric ulcers occurs through local injection of mixture of ferrous iron with ascorbic acid (Fe/ASA) solution into the gastric wall (Fulga *et al*., 2019). When given alone neither ferrous iron nor ascorbic acid able to cause gastric mucosal ulceration. Induction of ulcers in this model is a dose-dependent and causes ulcers that penetrate the muscularis mucosa. Generation of free radicals play a crucial role in the pathogenesis of ferrous iron-ascorbic acid-induced gastric ulcers (Fulga *et al*., 2019). Lipid peroxidation mediated by oxygen radicals known to cause oxidative deterioration of polyunsaturated lipids. This results in cell membrane destruction and cell damage which in turn leads to gastric mucosa ulceration and damage (Adinortey *et al*., 2013).

With regards to merits of this model, ulcers formed by this model resemble human gastric ulcers. Local injection of mixture of ferrous iron with ascorbic acid (Fe/ASA) solution into the gastric wall cause a penetrating ulcer that may cause perforation is a major limitation of this model.

1.4.5.15 Acetic acid-H. Pylori-induced ulcers

In this type of model, gastric ulcers is induced by administration of acetic acid into the subserosal layer of the glandular portion of the stomach followed by introduction of confirmed pathogenic strain of *H. pylori* intragastrically. A combined administration of acetic acid and *H. pylori* in this model is to induce chronic ulcers. This is because experimental induction of peptic ulcer by acetic acid only heals spontaneously but the introduction of *H. pylori* leads to a significant delay in ulcer healing (Bhattamisra *et al*., 2019).

H. pylori induces secretion of chemokines and pro-inflammatory cytokines such as IL-6, IL-8, IL-1 β, and TNF-α in gastric epithelial cells, which act as neutrophil-activating chemokines and lead to leukocyte infiltration. Increased leukocyte infiltration causes excessive generation of reactive oxygen species (ROS) which result in a damage to cell membranes and cellular components such as DNA, proteins, and lipids that finally leads to formation of gastric ulcers (Bhattamisra *et al*., 2019).

The merit of this model is that it is well validated model and ulcers formed by this method are similar to the human peptic ulcers in relation to their pathological characteristics and the healing process. Demerit related with model is that injection of acetic acid produced ulcers penetrating entire gastric wall & adherence of ulcer to liver.

The current study was done using indomethacin, ethanol and pyloric ligation induced gastric ulcer models. These models were selected among different ulcer models due to different reasons. One of the possible reasons is that they are the most accepted and preferred models for evaluation of anti-ulcer activity. The other reason is that since these agents are the most common causes of peptic ulcers in humans, they were preferably used to investigate the antiulcer activity of the extract. In addition, they were used to study the different mechanism of anti-ulcer activity of different drugs/ medicinal plants since they induce ulcer by different mechanisms.

2. OBJECTIVES

2.1 General objective

- To investigate anti-ulcer activity of aqueous root extract of *E. ventricosum* in experimental rats

2.2 Specific Objectives

- To investigate anti- ulcer activity of aqueous root extract of *E. ventricosum* against indomethacin-induced gastric ulcer in rats
- To investigate anti-ulcer activity of aqueous root extract of *E. ventricosum* against ethanol-induced ulcer in rats
- To investigate the anti- ulcer activity of aqueous root extract of *E. ventricosum* in pylori ligation-induced ulcers in rats.
- To determine the acute oral toxicity of aqueous root extract of *E. ventricosum* in rats

3. MATERIALS AND METHODS

3.1 Drugs and chemicals

Drugs and chemicals utilized in this study and obtained from vendors include indomethacin active ingredient 98% (Cadila pharmaceuticals P.L.C, Ethiopia), pantoprazole tablet (Deva Holding A.S. Istanbul, Turkey), tween 80% (CDC), Diethyl Ether AR/AS(Lobal Chemie), Formalaldehyde AR (Sigma Aldrich), phenolphthalein (Ranchem Industry, Turkey), Methyl orange (Dalul pharmaceuticals P.L.C, Ethiopia), sodium hydroxide assay 98.8% (Ranchem Industry, Turkey) and absolute ethanol 99% AR (Trust chemical laboratories). All the chemicals, reagents and drugs used in the study were of analytical grade.

3.2 Plant material

The roots of *E. ventricosum* were collected from its natural habitat around Chelia and Jibat districts of West Shewa Zone of Oromia Regional State, Central Ethiopia during a month of January, 2020. Identification and authentication of the plant specimens was done by a taxonomist and a voucher specimen (DH001) was deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University for future reference.

3.3 Extraction

The roots of the *E. ventricosum* was gently washed with tap water to remove dirt and soil & dried under shade at room temperature. After complete drying, plant samples were then finely ground using a mortar and pestle and packed in airtight plastic cups. A sample of 394.28g of dried and powdered roots were soaked in 3942.8ml of distilled water in conical flasks and extracted by boiling with distilled water (decoction) to simulate the traditional claims. The preparation was boiled in a water for 30 minutes until the water volume is halved. After being left to cool, it was filtered using nylon and gauze followed by whatman No.1 filter paper. Finally, the extract was subjected to lyophilization by freeze dryer to concentrate the extract. The yield of extract was 18.32%. Finally, the freeze-dried extract was kept in a refrigerator until used for the experiment.

3.4 Experimental animals

Healthy Sprague-Dawley rats of either sex, weighing 150–250g inbred at animal house of Pharmacology laboratory, School of Pharmacy, College of Health Sciences, Addis Ababa University, Ethiopia were used. Rats were housed in a plastic box cage under a room temperature and kept under 12/12 h light/dark cycle. The rats were allowed to feed standard pellets diet with free access to tap water. The animals were acclimatized to laboratory conditions for seven days

prior to the experiments. Animals were randomly divided into different groups (n= 6; male 3, female 3) for each of the models of the experiment and received treatments orally by oral gavage. The study was carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals and Organization of Economic Co-operation and Development (OECD) guidelines (Gelayee *et al*., 2017). Ethical approval from the Research Review Committee of School of Pharmacy was obtained.

3.5 Acute toxicity test

Acute toxicity study was performed according to Organization of Economic Co-operation and Development (OECD) 425 guidelines. It was carried out using the limit test dose of 2000 mg/kg and five female rats were used for the study. Rats were kept fasting overnight with free access to tap water prior to drug administration and 3-4 hours after administration of extract. Animals received the extracts sequentially and each rat received single dose 2000mg/kg of extract. The rat was observed 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 at least once during the first 30 min after dosing and then periodically during the first 24 h (with special attention during the first 4 h). Since no mortality was observed from the first rat during the first 24hours, other four rats were recruited and administered a single dose of 2000 mg/kg and were observed in the same manner and daily thereafter for a period of 14 days.

3.6. Pilot study

A pilot test was done to explore the efficacy of *E. ventricosum* on 20 rats which consisting of equal ratio of male to female against ethanol induced ulcer.

3.7 Gastric ulcer induced models

3.7.1 Indomethacin-induced gastric ulcers

Gastric ulcer was induced by administering indomethacin (25mg/kg) (Koriem *et.al*. 2015). After 24h of fasting with free access to water, rats were randomly assigned into five groups each consisting of six rats. Rats in the first group, served as control group and given orally distilled water (10 ml/kg) and rats in the second group (standard group) treated with pantoprazole (40mg/kg). Rats in the third, fourth and fifth groups received aqueous root extract of *E.*

ventricosum at doses of 100, 200 and 400mg/kg respectively. One hour after treatment, indomethacin (25mg/kg) was administered to all rats to induce gastric ulcer. Six hours after indomethacin administration, animals were sacrificed by cervical dislocation and the stomachs were removed, and opened along the greater curvature. The stomachs were gently rinsed with distilled water to remove the gastric contents & blood clots and examined for lesions. The ulcer scores, ulcer index and % ulcer protection were calculated.

Scoring of ulcers was made according to Datchanamurty *et al.* (2019) with slight modification

Normal stomach ..... …...….0

Red coloration..... …….....0.5

Spot ulcer.....……...1

Hemorrhagic streak……. 1.5

Number of ulcers <5……2

Number of ulcers >5……....3

Ulcer index (UI) was calculated by using the following formula (Sahoo *et al*. 2016)

$$UI = UN + US + UP \times 10\text{-}1$$

Where,

UI = Ulcer Index

UN = Average number of ulcers per animal

US = Average number of severity score

UP = Percentage of animals with ulcers.

The percentage of ulcer protection was determined as follows: -

$$\% \text{ Protective} = \frac{\text{control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

3.7.2 Ethanol-induced ulcer model

Gastric ulcer was induced by administration of absolute ethanol (99%). Animals were randomly divided into five groups of six animals each after 24h of fasting with free access of water. The first group represented the control group and received distilled water orally. Second group received pantoprazole (40 mg/kg) as standard drug. Third, fourth and fifth groups were given aqueous root extract of *E. ventricosum* in the dose of 100, 200 and 400mg/kg respectively. One hour after administration of extract of *E. ventricosum* and pantoprazole treatment, an absolute ethanol

(1ml/200g) was orally administered to each rat of every group (Bhoumik *et al*., 2016). One hour after alcohol administration, animals were sacrificed by cervical dislocation and stomachs were excised along the greater curvature, and bathed with distilled water to avoid residual matter on the ulcerated region. Mucosal damage was determined via scoring ulcer and ulcer index and % ulcer protection was also calculated using similar formula and scoring scheme done for indomethacin model. In addition, the volume, pH, free acidity and total acidity of gastric fluids were determined

Determination of volume and pH of gastric juice:

The gastric content of each stomach was collected and centrifuged for 10 min at 3000 rpm. The volume of supernatant and pH was measured using measuring cylinder and pH meter respectively.

Determination of total acidity

Total acidity and free acidity were determined according to Mary and Merina (2015). After gastric juice was collected and centrifuged for 10 min at 3000 rpm, 1ml gastric juice from the supernatant volume was diluted with 1ml of distilled water and taken into a 250 ml conical flask and three drops of phenolphthalein indicator was added to it 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 is expressed as mEq/L by the following formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality of NaOH}}{0.1} \times 100 \text{ Meq/L}$$

Determination of free acidity

After gastric juice was collected and centrifuged for 10 min at 3000 rpm, 1ml gastric juice from the supernatant volume was diluted with 1ml of distilled water and taken into a 250 ml conical flask and 3 drops of methyl orange was added and titrated with 0.01 N NaOH until all trace of the red color disappears and the color is yellowish orange. The volume of alkali added indicate free acidity. The free acidity was calculated as described earlier in total acidity.

3.7.3 Pyloric ligation-induced ulcer

Sprague-Dawley rats of either sex were randomly divided into five groups, each consisting of six rats. The animals were then fasted for a period of 24 hours with free access to water (Zewdu and Aragaw, 2020). Control group received distilled water orally (10ml/kg). Second group received pantoprazole (40 mg/kg) orally as a reference drug. Third, fourth and fifth groups were administered aqueous root extract of *E. ventricosum* in the doses of 100, 200 and 400mg/kg,

respectively. One hour after *E. ventricosum* and pantoprazole treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under ether anesthesia without causing any damage to the blood supply. After surgery (pyloric ligation) rats were deprived of food and water and allowed to recover and stabilize in individual cage during the postoperative period. Rats were sacrificed with excess of anesthetic ether after four hours of pyloric ligation and the stomachs were removed and incised along the greater curvature. Gastric damage was examined for severity of ulceration via scoring ulcer and ulcer index and % ulcer protection was also calculated as described above. The gastric juice was collected in falcon tube, centrifuged at 3000 rpm for 10 min and volume, pH, free acidity and total acidity of gastric juice were determined as described in ethanol-induced ulcer (Sahoo *et al*., 2016; Rai *et al*., 2015).

3.8 Histopathological study

In all the three models of ulcer studied, histopathological evaluations were conducted on gastric tissue samples collected. The stomach tissues from each group were fixed with 10% buffered formalin solution and dehydrated in graded alcohol. Tissue samples were cleared in xylene and embedded in paraffin wax in hot air oven (56°C). The specimens were then sectioned by microtome at a thickness of 5µm and stained with hematoxylin and eosin (HE) for histological analysis. Finally, the processed samples were examined by a pathologist under the microscope for histopathological changes such as hemorrhage, submucosal edema, epithelial erosion, and inflammatory cell infiltrates (Beiranvand and Bahramikia, 2020; Abdulla *et a*l., 2016). Extent of gastric mucosal ulceration were assigned a score using a histopathology scoring system (HSS) based on ulcer depth and degree of ulceration and were scored as follows (Yoo *et al*., 2018).

Score	Description
0	Normal intact mucosa
1	Slight surface erosive damages or small single or small multifocal lesions
2	Moderate mucosal damages with large single or large multifocal lesions or extensive superficial lesions
3	Severe total mucosal damages with extensive lesions with areas of apparent deep ulceration

3.9 Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS and one-way analysis of variance (ANOVA) followed by Tukey post-hoc test for multiple comparisons of the mean differences and responses of different treatment with controls and extract of varied doses. A “p” value less than 0.05 was considered significant.

4. RESULTS

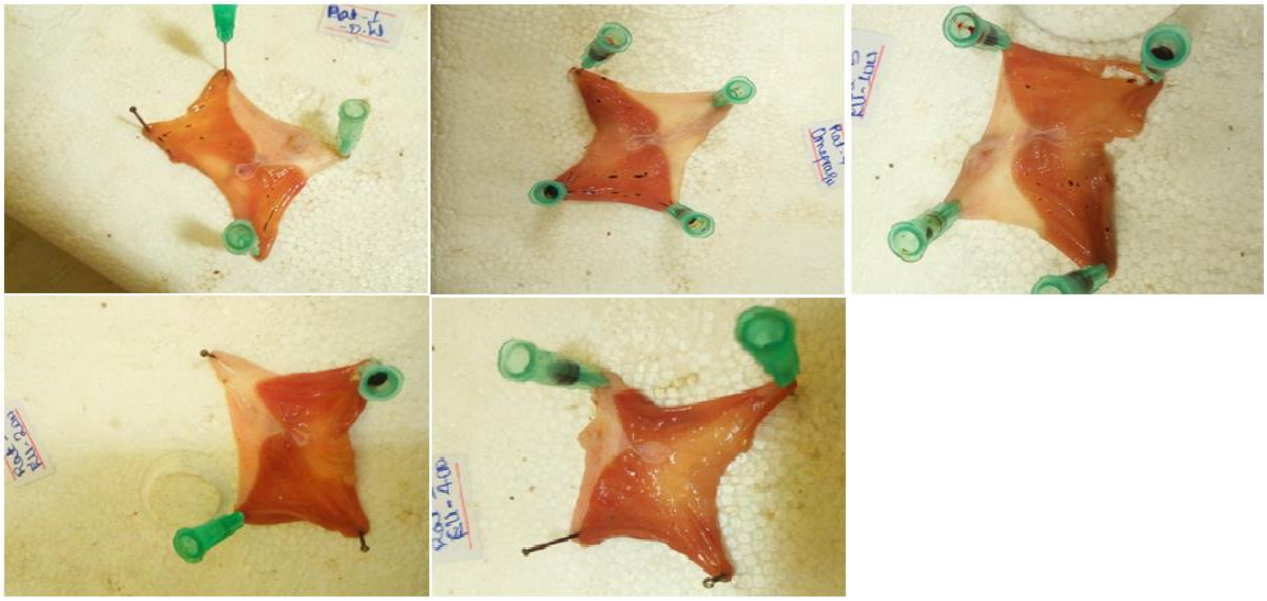
4.1 Acute oral toxicity study

Acute oral toxicity test performed in female rats indicated that the crude aqueous root extract of *E. ventricosum* did not cause any mortality up to a dose of 2000mg/kg. In addition, no sign and symptoms of toxicity related to behavioral, autonomic, neurologic, and physical profiles were observed after treatment with the extracts during the follow up period.

4.2 Pilot study

The pilot test was done on 20 rats with 4 rats per group and gastric ulcer was induced by administration of absolute ethanol. Gross examination of the dissected stomach showed that the negative control group produced prominent lesions of gastric mucosa. Similarly, omeprazole pretreated rats produced gross lesions in the gastric lumen of rats. Among the tested doses, oral pretreatment of aqueous root extract of *E. ventricosum* at a dose of 200 and 400mg/kg prevented gastric mucosal ulceration and showed improved protection with percentage of protection of 54.1% and 55.7% as compared with the control group.

Since ulcer protection was low (14.91%) when omeprazole used as standard drug in this pilot, another pilot test was done using pantoprazole as a reference drug. Oral pretreatment of pantoprazole given in the dose of 40mg/kg prevent gastric ulceration and showed a good ulcer protection on gross examination (Figure 2).



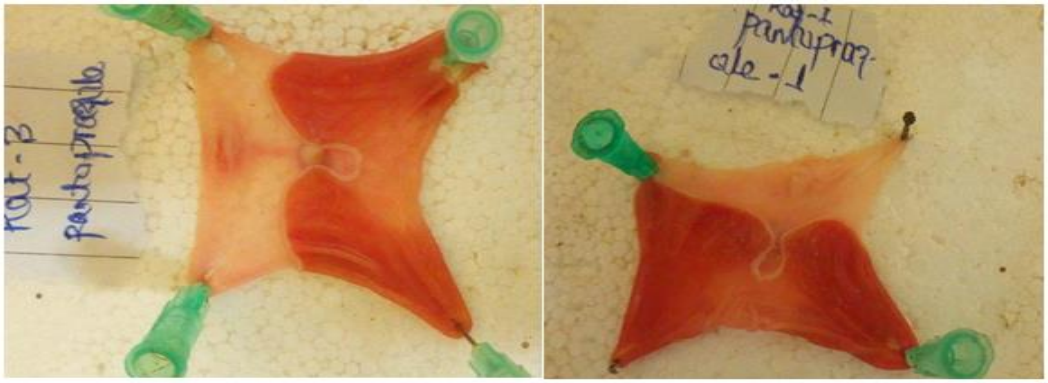


Figure 2: Gross effect of aqueous root extract of *E. ventricosum* against ethanol-induced ulcer in pilot test.

4.3 Effect of aqueous root extract of *E. ventricosum* on indomethacin-induced ulcer

As shown in Table 1, indomethacin induced a prominent ulceration in rats in control group as evidenced by a greater number of ulcers (20.00±2.42), severity score (7.35±0.25) and ulcer index (12.73). Pretreatment with aqueous root extract of *E. ventricosum* significantly ($p<0.001$) decreased number of ulcers 6.33± 2.38, 3.67±1.20 and 1.00±.68 at a dose of 100, 200 and 400mg/kg, respectively. *E. ventricosum* given in the dose of 400mg/kg significantly ($p<0.05$) decreased the severity score of ulcers when compared to low dose of the extract (EV-100). Gastric gross mucosal lesions induced by indomethacin are indicated in Figure 3.

Table 1. Effect of aqueous root extract of *E. ventricosum* on ulcer index and percentage of protection against indomethacin-induced ulcer

Treatment group	Number of ulcers	Severity score	Ulcer index	Percentage protection (%)
Control (DW)	20.00±2.42	7.35±0.25	12.73	_
Standard 40	0.00±0.00 [1b]	0.83±0.83[1a]	0.08	99.3
EV-100	6.33± 2.38[1b]	4.75±1.31	9.44	25.8
EV-200	3.67±1.20[1b]	3.17±.76[1a]	7.35	42.3
EV-400	1.00±.68[1b]	1.67±.42[1a, 2a]	3.60	71.7

Data are expressed as mean ± SEM for each group (N= 6). The results are compared [1]against control, [2]against EV-100. [a] p<0.05; [b]p<0.001. Control = distilled water, standard= pantoprazole, EV= *Ensete ventricosum*, numbers (40,100,200, 400) indicate doses in mg/kg.

As shown in Figure 3, gross examination of indomethacin-induced ulcerated rats showed that rats in control group caused marked ulceration. Pretreatment of rats with aqueous root extract of *E. ventricosum* at a dose of 100, 200 and 400mg/kg produced a dose dependent decrement in formation of ulcers.

Figure 3: Gross effect of aqueous root extract of *E. ventricosum* against indomethacin-induced gastric ulcer in rats.

4.4 Histopathological effect of aqueous root extract of *E. ventricosum* against indomethacin-induced gastric ulcer in rats

Rats in the negative control group pretreated with distilled water showed multifocal erosion in the mucosal epithelium and epithelial necrosis in the gastric mucosa. Pantoprazole treated rats showed intact gastric mucosa with no histological changes. Rats pretreated with aqueous root extract of *E. ventricosum* at a dose of 100mg/kg revealed focal erosion in the mucosal epithelium whereas as

rats treated with aqueous root extract of *E. ventricosum* at a dose of 200 and 400mg/kg revealed normal gastric mucosa (Figure 4).

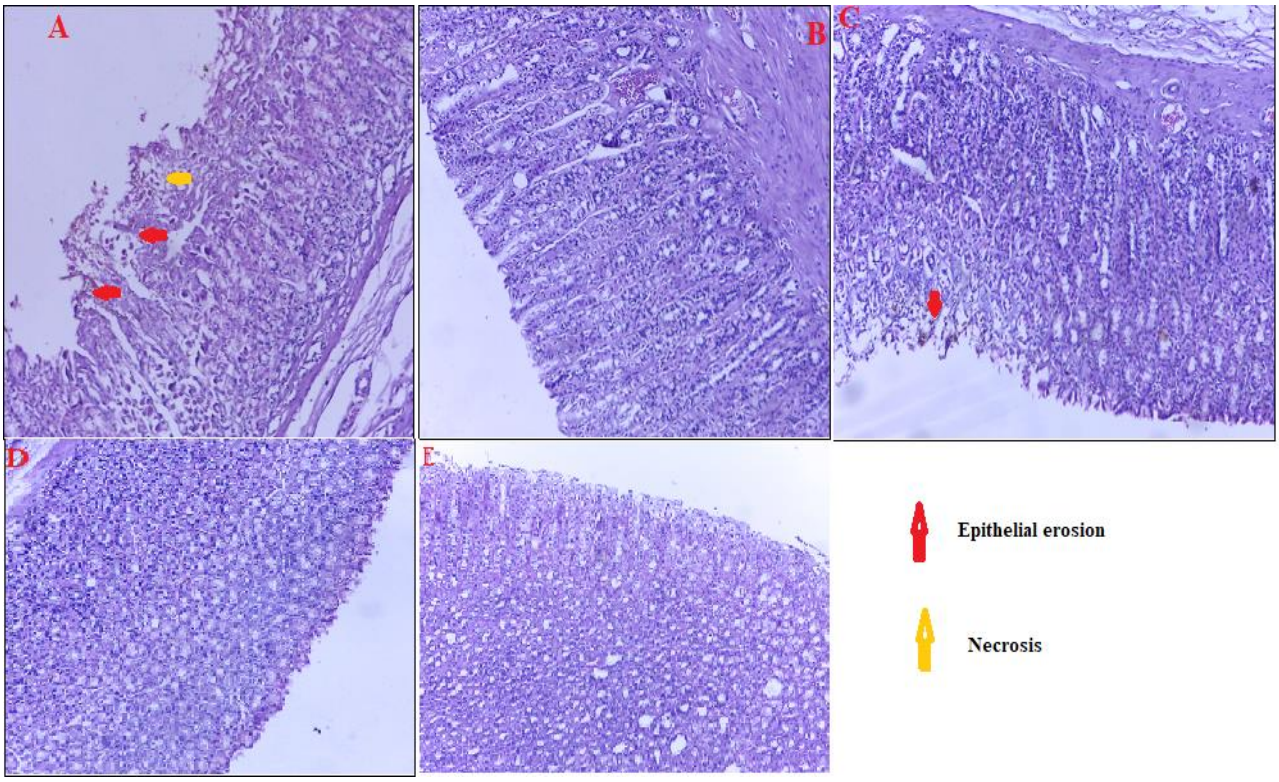


Figure 4: Histopathological effect of aqueous root extract of *E. ventricosum* against indomethacin-induced gastric ulcer model in rats. (A) Negative control group received distilled water; (B) positive control group received pantoprazole; (C) rats pretreated with 100 mg/kg of E. ventricosum; (D) rats pretreated with 200 mg/kg of E. ventricosum; (E) rats pretreated with 400 mg/kg of E. ventricosum.

4.5 Effect of aqueous root extract of *E. ventricosum* against ethanol-induced ulcer

Oral administration of absolute ethanol caused prominent gastric lesion in experimental rats with markedly increased number of ulcers, severity score and ulcer index (17.17±1.60, 7.83±0.11 and 12.50) respectively. Administration of aqueous root extract of *E. ventricosum* prior to ethanol significantly ($p<0.001$) reduced number of ulcers in all treatment groups. *E. ventricosum* at a dose of 200 and 400mg/kg significantly ($p<0.05$) decreased severity score of ulcers when compared with control group (Table 2).

As shown in Table 3, Pretreatment with aqueous root extract of *E. ventricosum* given in the dose of 100, 200 and 400mg/kg reduced volume of gastric juice significantly ($p<0.05$) by 3.05± 0.13,

2.15± 0.24, and 1.11± 0.10 respectively with percentage of inhibition of 18.67%, 42.67% and 70.40% when compared to 3.75± 0.08 in control group. *E. ventricosum* given in the dose of 200mg/kg significantly ($p<0.05$) reduced the volume of gastric juice as compared to 100mg/kg of the extract. Similarly, *E. ventricosum* at a dose of 400mg/kg significantly ($p<0.05$) reduced the volume of gastric juice as compared to 100 and 200mg/kg of *E. ventricosum*.

As shown also in Table 3, animals treated with aqueous root extract of *E. ventricosum* given at a dose of 200 and 400mg/kg significantly ($p<0.05$) increased pH of the gastric juice when compared to 1.08±0.11 in control group. *E. ventricosum* given in the dose of 200mg/kg significantly ($p<0.05$) increased pH of the gastric juice as compared to 100mg/kg of *E. Ventricosum*. Similarly, *E. ventricosum* at a dose of 400mg/kg significantly ($p<0.05$) increased pH of the gastric juice as compared to 100 and 200mg/kg of *E. ventricosum*.

As shown also in Table 3, oral administration of absolute ethanol caused an increase in total acidity and free acidity. Pretreatment of animals with the extract significantly ($p<0.001$) decreased both total acidity and free acidity in all treatment groups when compared to the vehicle control group. *E. ventricosum* at a dose of 400mg/kg significantly ($p<0.001$) decreased free acidity as compared to 100 and 200mg/kg of *E. ventricosum*.

Table 2. Effect of aqueous root extract of *E. ventricosum* on ulcer index and percentage of protection against ethanol-induced ulcer

Treatment group	Number of ulcers	Severity score	Ulcer index	Percentage protection (%)
Control (DW)	17.17±1.60	7.83±0.11	12.50	_
Standard 40	1.50±1.02[1b]	2.25±0.57[1a]	3.71	70.32
EV-100	5.50±1.75[1b]	5.83±1.12	9.47	24.24
EV-200	3.17±1.40[1b]	3.17±0.53[1a]	7.30	41.60
EV-400	2.83±1.33[1b]	3.33±1.06[1a]	5.62	55.04

Data are expressed as mean ± SEM for each group (N= 6). The results are compared [1]against control; [a] p<0.05; [b]p<0.001. Control = distilled water, standard= pantoprazole, EV= *Ensete ventricosum*, numbers (40,100,200, 400) indicate doses in mg/kg.

Gross examination of the dissected stomach of ethanol-induced ulcer showed that the negative control group produced gross ulceration of gastric mucosa. Prior administration of aqueous root extract of *E. ventricosum* at a dose of 100, 200 and 400mg/kg caused a decrement in formation of ulcer and showed anti-ulcer activity (Figure 5).

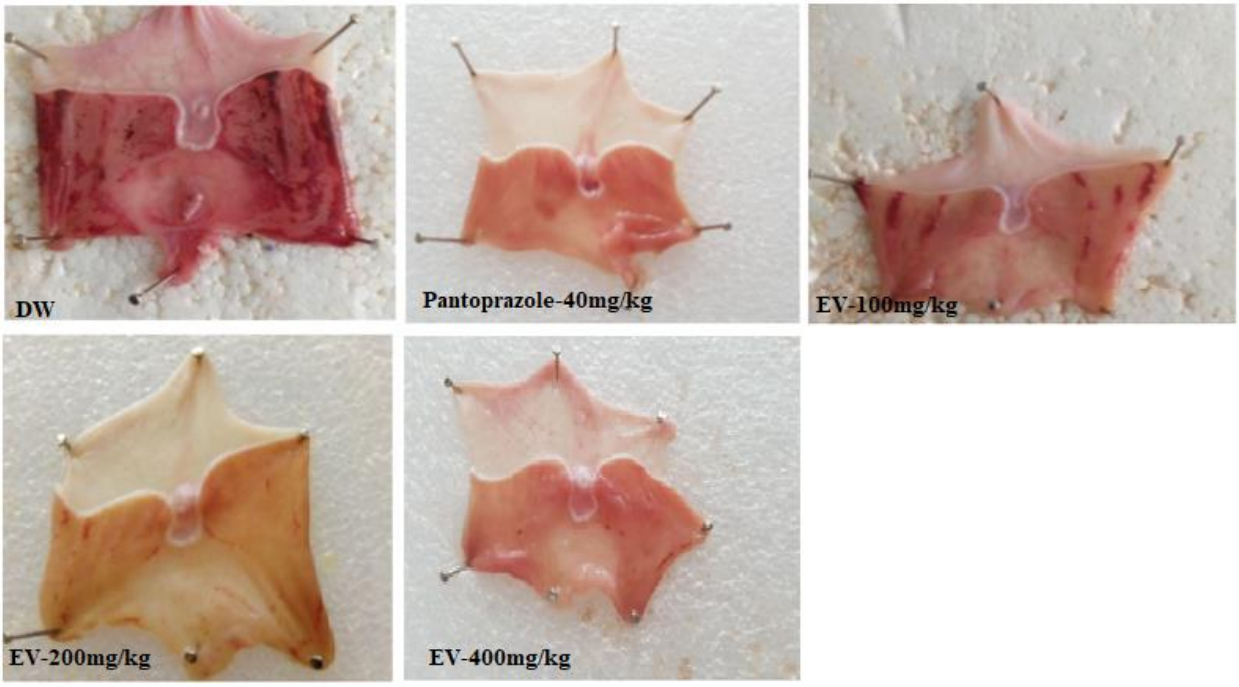


Figure 5: Gross effect of aqueous root extract of *E. ventricosum* on ethanol-induced gastric ulceration in rats.

4.6 Histopathological effect of aqueous root extract of *E. ventricosum* on ethanol-induced gastric mucosal lesions

Exposure of rats to ethanol caused comparatively extensive damage to the gastric mucosa with severe and deep epithelial cells damage, mild leucocytes infiltration of the mucosa and submucosal layer and slight hemorrhage. Rats that received pretreatment with pantoprazole showed mild focal erosions in the mucosal epithelium. Pretreatment with aqueous root extract of *E. ventricosum* given in the dose of 100mg/kg revealed multifocal superficial epithelial erosions. On the other hand, in the dose of 200 and 400mg/kg the extract showed focal epithelial erosions and mild surface epithelial erosions where most of the mucosal part of the stomach were preserved respectively.

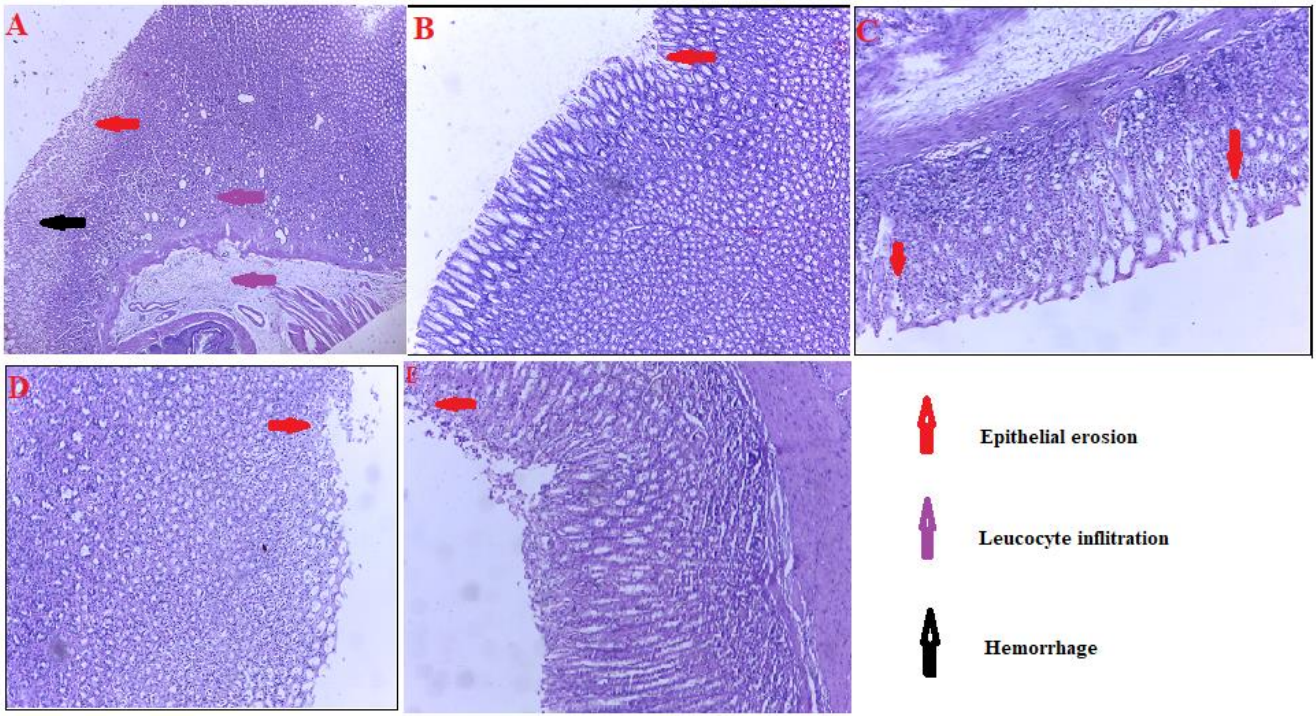


Figure 6: Histopathological effect of aqueous root extract of *E. ventricosum* on ethanol-induced gastric mucosal lesions. (A) Negative control group received distilled water; (B) positive control group received pantoprazole; (C) rats pretreated with 100 mg/kg of E. ventricosum; (D) rats pretreated with 200 mg/kg of E. ventricosum; (E) rats pretreated with 400 mg/kg of E. ventricosum.

Table 3. Effect of aqueous root extract of *E. ventricosum* on volume, pH, total and free acidity of gastric juice in ethanol-induced ulcer

Treatment group	Volume of gastric juice (ml)	Percentage volume inhibition (%)	pH of gastric juice	Total acidity (mEq/L)	Free acidity (mEq/L)
Control (DW)	3.75± 0.08	-	1.08±0.11	590.00± 14.14	486.67±9.89
Standard 40	1.02± 0.12[1a]	72.80	5.900± 0.29[1a]	386.67± 10.54[1b]	236.67±8.82[1b]
EV-100	3.05± 0.13[1a]	18.67	1.52± 0.11	461.67±11.37[1b]	402.50±10.47[1b]
EV-200	2.15± 0.24[1a,2a]	42.67	3.30± 0.13[1a,2a]	430.00± 9.31[1b]	358.33±11.38[1b]
EV-400	1.11± 0.10[1a, 2a, 3a]	70.40	5.03± 0.17[1a, 2a,3a]	390.00± 15.28[1b,]	280.00±9.66[1b, 2b,3b]

Data are expressed as mean ± SEM for each group (N= 6). The results are compared [1]against control, [2]against EV-100, [3]EV-200. [a]p<0.05; [b]p<0.001. Control = distilled water, standard= pantoprazole, EV= *Ensete ventricosum*, numbers (40,100,200, 400) indicate doses in mg/kg. mEq/L= miliequivalents per liter

4.7 Effect of aqueous root extract of *E. ventricosum* against pyloric ligation-induced ulcer

As shown in Table 4, aqueous root extract of *E. ventricosu*m at doses of 200 and 400mg/kg significantly ($p<0.05$) reduced both ulcers number and severity score compared to the vehicle control group. Pantoprazole exhibited a significant ($p<0.05$) reduction in both ulcer numbers and severity score of ulcers when compared with control group.

Pretreatment of animals with aqueous root extract of *E. ventricosum* at a dose of 200 and 400mg/kg significantly ($p<0.001$) reduced the volume of gastric juice compared to the vehicle control group with percentage of inhibition of 40.60% and 57.52% respectively

As shown also in Table 5, administration of aqueous root extract of *E. ventricosum* at a dose of 200 and 400mg/kg significantly ($p<0.001$) increased the pH of gastric juice compared to vehicle control group. *E. ventricosum* at a dose of 400mg/kg significantly ($p<0.001$) increased pH of the gastric juice as compared to 100mg/kg of *E. ventricosum*.

Pretreatment of animals with aqueous root extract of *E. ventricosum* significantly ($p<0.001$) decreased both total acidity and free acidity in all treatment groups when compared to vehicle control group (Table 5). *E. ventricosum* at a dose of 200 and 400mg/kg significantly ($p<0.001$) reduced free acidity while *E. ventricosum* given in the dose of 400mg/kg significantly ($p<0.001$) reduced total acidity as compared to 100mg/kg of *E. ventricosum*. Treatment with pantoprazole considerably reduced both total acidity and free acidity when compared to the control group ($p<0.001$).

Table 4. Effect of aqueous root extract of *E. ventricosum* on ulcer index and percentage of protection in pyloric ligation-induced ulcer

Treatment group	Number of ulcers	Severity score	Ulcer index	Percentage protection (%)
Control (DW)	1.67±0.71	3.58±0.47	8.86	_
Standard 40	0.00±0.00[1a]	0.25±0.17[1a]	0.03	99.60
EV-100	0.67±0.33	2.42±0.64	5.31	40.07
EV-200	0.17±0.17[1a]	1.58±0.30[1a]	3.51	60.38
EV-400	0.00±0.00[1a]	1.33±0.25[1a]	0.13	98.53

Data are expressed as mean ± SEM for each group (N= 6). The results are compared [1]against control; [a]$p<0.05$. Control = distilled water, standard= pantoprazole, EV= *Ensete ventricosum*, numbers (40,100,200, 400) indicate doses in mg/kg.

As shown in Figure 7, macroscopic examination of pyloric ligation-induced ulcerated rats showed that rats in vehicle control group produced relatively a gross ulceration of the gastric mucosa. Pretreatment with aqueous root extract of *E. ventricosum* at a dose of 100, 200 and 400mg/kg caused a decrement in formation of ulcer and showed gastroprotective effect.

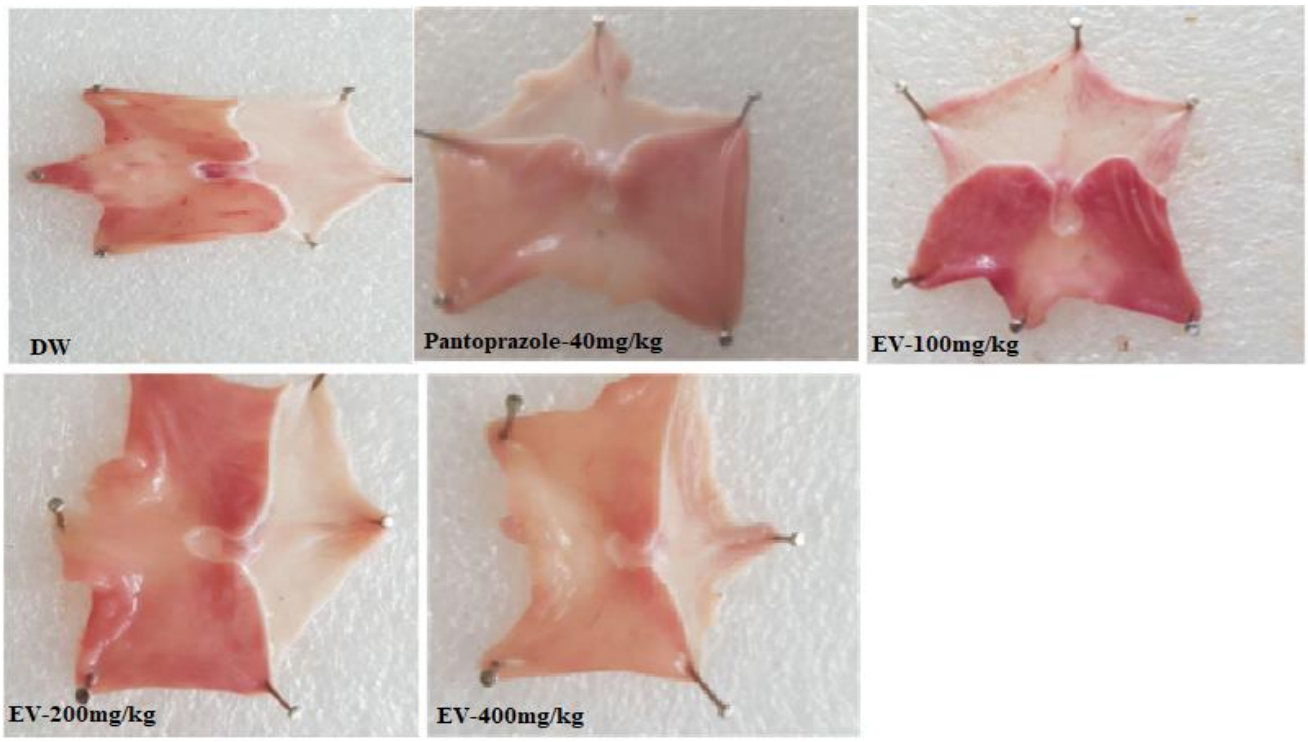


Figure7: Gross effect of aqueous root extract of *E. ventricosum* against pyloric ligation-induced gastric ulceration in rats

4.8 Histopathological effect of aqueous root extract of *E. ventricosum* on pyloric ligation-induced gastric mucosal lesions

Histopathological observation of pyloric ligation-induced gastric lesions in ulcer control group showed comparatively a multifocal erosion of the epithelial membranes of the gastric mucosa and loss of the mucosal layer thickness. Rats pretreated with aqueous root extract of *E. ventricosum* at a dose of 100mg/kg revealed focal superficial erosion. On the other hand, pre-treatment with *E. ventricosum* given in the dose of 200 and 400mg/kg as well as positive control group showed normal structure of the epithelium of the gastric mucosa (Figure 8).

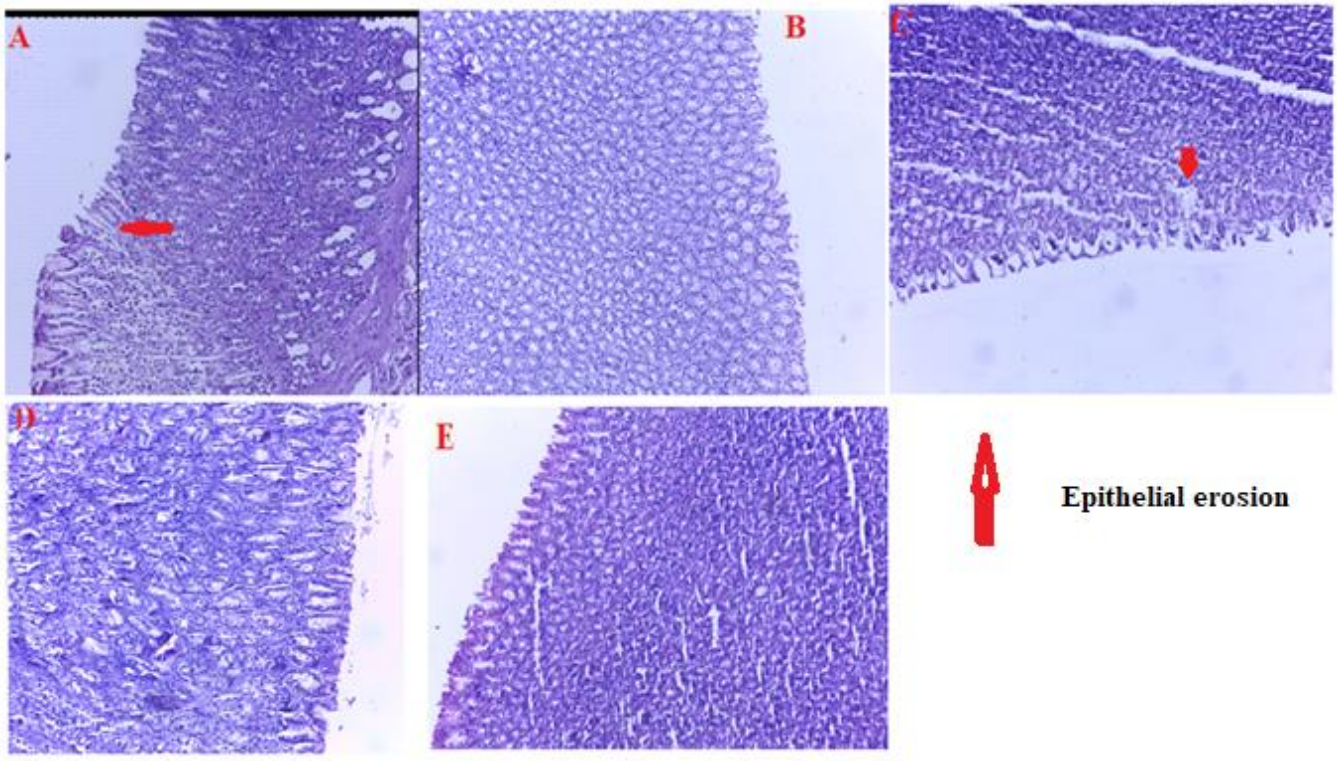


Figure 8 Histopathological effect of aqueous root extract of *E. ventricosum* on pyloric ligation-induced gastric mucosal lesions. (A) Negative control group received distilled water; (B) positive control group received pantoprazole; (C) rats pretreated with 100 mg/kg of E. ventricosum; (D) rats pretreated with 200 mg/kg of E. ventricosum; (E) rats pretreated with 400 mg/kg of E. ventricosum.

Table 5. Effect of aqueous root extract of *E. ventricosum* on volume, pH, total and free acidity of gastric juice in pyloric ligation- induced ulcer

Treatment group	Volume of gastric juice (ml)	Percentage volume inhibition (%)	pH of gastric juice	Total acidity (mEq/L)	Free acidity (mEq/L)
Control (DW)	4.12±0.34	-	1.57±0.15	725.00±16.17	546.00±10.42
Standard 40	1.53±0.27[1b]	62.86	4.33±0.20[1b]	430.00±9.31[1b]	146.67±18.01[1b]
EV-100	3.33±0.33	19.17	2.22±0.25	613.33±22.61[1b]	441.67±24.96[1b]
EV-200	2.20±0.27[1b]	40.60	2.97±0.19[1b]	545.00±7.64[1b]	223.33±6.67[1b, 2b]
EV-400	1.75±0.25[1b]	57.52	3.52± 0.14[1b,2b]	476.67±8.82[1b,2b]	188.33±9.46[1b, 2b]

Data are expressed as mean ± SEM for each group (N= 6). The results are compared [1]against control, [2]against EV-100. [b]p<0.001. Control = distilled water, standard= pantoprazole, EV= *Ensete ventricosum*, numbers (40,100,200, 400) indicate doses in mg/kg. mEq/L= miliequivalents per litre

4. DISCUSSION

The present study has evaluated antiulcer effects of aqueous root extract of *E. ventricosum* against indomethacin, ethanol and pyloric ligation-induced gastric ulcers in rats. The results of this study conclusively showed that the aqueous root extract of *E. ventricosum* showed significant antiulcer activity against all models studied.

Administration of aqueous root extract of *E. Ventricosum* caused no mortality at a dose of 2000 mg/kg within 24 hours and for the following 14 days. In addition, the extract did not cause any sign and symptoms of toxicity related to behavioral, autonomic, neurologic, and physical profiles. These data suggest that median lethal dose of aqueous root extract of *E. ventricosum* is quite above 2000 mg/kg.

Non-steroidal anti-inflammatory drugs (NSAIDS) which are mostly employed for management of fever, pain and inflammation have been widely used in medical practice throughout the world. Among NSAIDS, indomethacin is a potent agent with higher ulcerogenic potential both in humans and experimental animals (Akpamu *et al*., 2013). Hence, it is a preferred agent for induction of ulcer in experimental animals and the result confirmed high potential in induction of ulcer (Akpamu *et al*., 2013).

In the present study 25mg/kg of indomethacin was used for ulcer induction and it caused a marked and visible gastric mucosal ulceration in exposed experimental rats in the control group as evidenced by a greater number of ulcers, severity score and ulcer index. Moreover, multifocal erosion in the epithelium layer of the mucosa and epithelial necrosis in the gastric mucosa was observed in the negative control group treated with indomethacin as confirmed by histopathological findings. Pretreatment with aqueous root extract of *E. ventricosum* significantly reduced level of gastric mucosal ulceration and improved ulcer protection. Furthermore, histopathological findings of rats pretreated with aqueous root extract of *E. ventricosum* at a dose 200 and 400 mg/kg also showed normal gastric mucosal structure; thus, the extract displayed significant anti-ulcerogenic effect.

The antiulcerogenic effect of the plant against indomethacin-induced gastric ulcer might be due to the presence of secondary metabolites like flavonoids, phenols, saponins, and tannins in the aqueous root extract that were reported to have gastroprotective activity in different experimental studies (Mekonnen *et al*., 2020; salwe *et al*., 2020; Zaghlool *et al*., 2019; Oluwabunmi and Abiola, 2015). Tannins may prevent ulcer development through protecting the outermost layer of the

gastric mucosa and enable the gastric mucosa to resist to chemicals and mechanical injury by changing the mucosal structure (Mohd *et al*., 2011). Flavonoids and other phenolic compounds possess antiulcer effect due to their enhancing activity on the protective factors via synthesis of PGE_2, increasing capillary resistance and improving microcirculation (Mekonnen *et al*., 2020; Rai *et al.*, 2015). In addition, flavonoids and tannins exerts cytoprotective effect in indomethacin-induced ulcer due to their potent antioxidant activities by promoting antioxidant enzymes synthesis (SOD and GSH), and inhibiting reactive oxygen species (Rai *et al.*, 2015).

In this study, the antiulcer effect of the aqueous root extract of the plant was also investigated against ethanol-induced gastric ulcers. Ethanol-induced gastric ulcer is also one of the most common and preferred ulcer model used to evaluate of gastro-protective activity of plant materials in experimental animals (Sidahmed *et al.*, 2019). The mechanisms by which ethanol causes gastric ulcer are multifactorial; one of which is by direct toxic effect on the gastric mucosa which leads to gastric lesions, edema, inflammation, hemorrhage and necrosis in the mucosal membrane of the gastric (Akhtar *et al*., 2010). Induction of ulcer by ethanol is also mediated through the hematogenous route which causes disturbance in the gastric circulation thereby contributing to stasis in gastric blood flow (Suralkar and Gund, 2014; Akhtar *et al.*, 2010). Another mechanism by which ethanol-induced gastric ulcer in experimental animals is through inhibition of the defensive factors (reducing prostaglandins synthesis, secretion of bicarbonate and production of mucus) and increased intracellular membrane permeability to sodium and water due to cell and plasma membrane damage which may subsequently lead to cell lysis (Suralkar and Gund, 2014).Thus, it leads to the development of the hemorrhage and necrosis in the damaged gastric mucosal areas which results in increased ulcer score and ulcer index.

In this study, administration of absolute ethanol to experimental animals significantly resulted in a gross gastric lesion with markedly increased number of ulcers, severity score and ulcer index. In addition, histopathological examinations revealed extensive damage to the gastric mucosa with severe and deep epithelial cells damage, mild leucocytes infiltration of the mucosa and submucosal layer and slight hemorrhage. Prior administration of aqueous root extract of *E. ventricosum* offered significant protection against damage of gastric mucosa induced by the ethanol and improved ulcer protection. Moreover, histopathological findings showed focal epithelial erosions and mild surface epithelial erosions where most of the mucosal part of the stomach preserved. This gastroprotective effect of aqueous root extract of *E. ventricosum* might be due to its effects in augmenting the mucus

and bicarbonate secretion, stimulation of the PG synthesis, decrease in intracellular membrane permeability and reducing direct toxic effect of ethanol, and mediated through tannins, flavonoids and saponins contained in the extract. Tannins prevent ethanol-induced gastric irritation, hemorrhage and cell and plasma membrane damage through rendering the direct toxic effect to the outermost layer of the mucosa and the permeability of outermost layer of the mucosa by causing precipitation of mucosal proteins and promoting the healing effect (Yismaw *et al*., 2020; Hussain *et al*., 2015). In addition, saponins may contribute to the antiulcer activity via facilitating the synthesis of the PG in the gastric mucosa (Yismaw *et al*., 2020; Inas *et al*., 2011). Furthermore, flavonoids act as gastroprotective by promoting mucus production and increasing capillary resistance and increases blood flow by enhancing the synthesis of prostaglandins (Devaraj *et al*., 2011).

The main aggressive mechanisms by which oral administration of ethanol caused peptic ulcer is through enhancing the process of lipid peroxidation in response to abnormal elevation of reactive oxygen species (Katary and Salahuddin, 2017; Hussain *et al*., 2015). Exposure to ethanol induces inhibition of the antioxidant enzyme like GSH, CAT and SOD while provokes free radicals generation that leads to deleterious effects in the integrity and function of the mucous membrane (Katary and Salahuddin, 2017). The metabolism of ethanol causes the release of superoxide anion and hydroperoxy free radicals that have been found to be involved in the mechanism of acute and chronic peptic ulcer disease (Suralkar and Gund, 2014).

Oral pre-treatment of experimental rats with aqueous root extract of *E. ventricosum* at a dose of 200 and 400mg/kg produced gastroprotective effects when compared with the control group. The observed gastroprotective effects of the extract might be due to the secondary metabolites found in the extract. It is well known that flavonoids like quercetin, kampferol and rutin are special metabolites which are usually known with their strong antioxidant activity that efficiently remove superoxide anion, hydroperoxy and hydroxyl radicals and involved in cytoprotection against gastric mucosal ulceration. In addition, coumarins have been reported to exhibit antioxidant activity which might be responsible for its anti-ulcer effect (Rancy and Krishnakumari, 2015).

Pyloric ligation-induced gastric ulcer model is generally used to study the antiulcer activity of plant extract. Ligation of the pyloric end of the stomach is an important method to determine the degree of ulceration in exposed rats through measuring the mean ulcer score and ulcer index (Hussain *et al*., 2015). Induction of gastric ulcer in this model are probably mediated by stress

induced secretion of HCl in excess amounts from the parietal cells, histamine release from enterochromafin like cells which facilitate acid secretion and a reduction in mucus production that impairs the gastroprotective effect (Gelayee *et al.,* 2017; Hussain *et al*., 2015). Development gastric ulcer by the pyloric ligation is caused by increased volume of gastric secretion and accumulation of gastric acid that leads to the impairment of the gastric mucosal barrier (Akhatar *et al*., 2010). Gastric mucosal damage is also due to an auto digestion of the gastric mucosal membrane in pyloric ligation-induced ulcer (Mekonnen *et al*., 2020). Studies have shown that pyloric ligation-induced ulcer may also be associated with generation of free radicals as revealed by changes in the antioxidant status (Gelayee *et al*., 2017).

In the present study, a marked gastric ulceration was observed in pyloric ligation-induced ulcer in control group. In addition, histological observation of pyloric ligation -induced gastric lesions in ulcer control group showed comparatively a multifocal erosion of the epithelial membranes of the gastric mucosa and loss of the mucosal layer thickness. Prior administration of aqueous root extract of *E. ventricosum* given in the dose of significantly reduced both ulcers number and severity score when compared to the vehicle control group and histopathological findings revealed normal structure of the epithelium of the gastric mucosa. Moreover, pretreatment of these rats with aqueous root extract of *E. ventricosum* exhibited a reduced mean ulcer index with best ulcer protection in pyloric ligation-induced models with 98% protection at 400mg/kg dose. The gastroprotective effect of the extract might be due to the phytochemical constituents found in it. Flavonoids have potential cytoprotective effect since they are effective antioxidants due to their potent free radical scavenging properties (Inas *et al*., 2011). In addition, flavonoids have cytoprotective activities since they increase the synthesis of prostaglandins, secretion of bicarbonates and mucus production (Yismaw *et al*., 2020; Dashputre *et al*., 2011).

It is well known that increased gastric acid secretion plays an important role in the etiology and progression of the gastric ulcer (Ibrahim *et al*., 2018). Administration of ethanol increases acid secretion principally by stimulating the secretion of gastrin, histamine release from enterochromafin like cells and to a lesser extent by a direct effect on the gastric parietal cells (Mary and Merina, 2015). In addition, pyloric ligation caused massive accumulation of gastric acid which is probably mediated by stress induced secretion of HCl in excess amounts from the parietal cells, histamine release from enterochromafin like cells (Gelayee *et al.,* 2017). Increased gastric acid

secretion results in decreased prostaglandins synthesis that leads impaired gastro-protection (Ibrahim *et al*., 2018).

The results of the present study showed that consumption of ethanol and ligation of the pyloric end of the stomach resulted in a significant increase in the volume of the gastric juice as well as free and total acidity in the vehicle control group. Prior administration of aqueous root extract of *E. ventricosum* decreased volume of the gastric acid with concomitant decreased in the free and total acidity. Thus, the extract possesses dose dependent antisecretory and anti-ulcerogenic activity. The observed antisecretory activities may be attributed to the phytochemical constituents of the extract. Glycosides have been shown to have antisecretory activity through inhibition the gastric acid secretion (Akhtar *et al*., 2010). In addition, flavonoids have potential antisecretory effect due to it inhibit H+/K+-ATPase and decrease histamine secretion from mast cells by inhibition of histidine decarboxylase (Yismaw *et al*., 2020; Gelayee *et al*., 2017; Rai *et al.*, 2015).

Another factor responsible for the pathogenesis of ulcer and gastric damage in experimental animals is pH of the gastric juice. Low pH is a manifestation of elevated concentration of the hydrogen ion which is an aggressive factor facilitating damage of the gastric mucosa (Elsayed *et al.*, 2019). In the present study, administration of ethanol and pyloric ligation caused a reduction in gastric pH level in control group. Pre-treatment with aqueous root extract of *E. ventricosum* given in the dose of 200 and 400mg/kg significantly improved gastric pH levels in comparison to vehicle control group. The extract produced dose dependent increased in pH of the gastric juice. The efficiency of aqueous root extract of *E. ventricosum* in increasing gastric pH thereby decreasing the gastric ulcer might be attributed due to the presence of flavonoids in the extract. Flavonoids are important compounds that have been shown to exhibit antisecretory properties by rising pH of gastric juice (Elsayed *et al*., 2019).

The limitation of the study was unavailability of topers reagent; instead methyl orange was used for titration.

6. CONCLUSION

From the present study, it can be concluded that crude aqueous root extract of *Ensete ventricosum* displayed significant antiulcer activity against indomethacin, ethanol and pyloric ligation-induced ulcer models which confirms the traditional use of the plant. The extract also possessed good safety profile which collectively indicates its potential for the development of new agent for the management of peptic ulcer disease.

7. RECOMMENDATION

Based on the results of the present study, the following are recommended to be investigated further

- Sub-acute and chronic toxicity of the plant extract should be conducted
- Mechanism of antiulcer activity of the extract should be determined
- Isolation and identification of bioactive compounds responsible for antiulcer activity of the extract should be done

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