Department of Pharmacology and Clinical Pharmacy

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Addis Ababa University

Evaluation of gastroprotective activities of aqueous and 80% methanol leaf extracts of *Stephania abyssinica* (Quart. -Dill. & A. Rich.) Walp. (Menispermaceae) in rats

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This is to certify that the thesis prepared by Banchayehu Firehun, entitled: "**Evaluation of gastroprotective activities of aqueous and 80% methanol leaf extracts of *Stephania abyssinica* (Quart. -Dill. & A. Rich.) Walp. (Menispermaceae) in rats**", and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Evaluation of gastroprotective activities of aqueous and 80% methanol leaf extracts of *Stephania abyssinica* (Quart. -Dill. & A. Rich.) Walp. (Menispermaceae) in rats

Banchayehu Firehun

Addis Ababa University, 2023

Peptic ulcer disease is the most common gastrointestinal tract disorder that mainly affects the stomach and proximal duodenum, and occurs when the protective and damaging factors are out of balance. Its therapy is challenging due to the rise of *Helicobacter pylori* resistance, adverse effects from current medications, and its complications. This calls for the development of effective and safe gastroprotective agents from alternative sources such as medicinal plants. *Stephania abyssinica* is a medicinal plant used for the treatment of gastritis in Ethiopia, but there is no scientific investigation. Thus, the aim of this study was to evaluate the gastroprotective activities of both aqueous and 80% methanol leaf extracts of *Stephania abyssinica* in experimental rats. Decoction and maceration techniques were used to prepare aqueous and 80% methanol leaf extracts, respectively. The extracts were evaluated against pyloric ligation, indomethacin, and ethanol-induced gastric ulcer models at doses of 100, 200, and 400 mg/kg. Negative control received 2% tween 80, while positive controls received 20 mg/kg of omeprazole and 100 µg/kg of misoprostol. In the pyloric ligation induced gastric ulcers, all doses of both extracts significantly reduced the ulcer index and gastric juice volume, while doses of 200 and 400 mg/kg exhibited a significant increase in mucus content and gastric juice pH as well as decrease in free and total acidity as compared to negative control. In indomethacin and ethanol induced gastric ulcers, pretreatment with both extracts significantly reduced the ulcer index and enhanced gastric mucin content in a dose dependent manner. Phytochemical screening of extracts showed the existence of flavonoids, phenols, tannins, saponins, alkaloids, and coumarins with high contents of alkaloids, phenols, and flavonoids in methanol extract. The findings indicated that the leaves of *Stephania abyssinica* possessed remarkable gastroprotective activities against experimentally induced gastric ulcers. This possibly justify the traditional use of *Stephania abyssinica* leaves to treat gastritis.

Key words: Peptic ulcer disease, *Stephania abyssinica*, gastroprotective activity, pyloric ligation induced gastric ulcer, indomethacin induced gastric ulcer, ethanol induced gastric ulcer

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Abbreviations and acronyms

AAU	Addis Ababa University
ACh	Acetylcholine
AE	Atropine equivalent
ANOVA	Analysis of variance
AQ	Aqueous extract
BCG	Bromocresol green
CagA	Cytotoxin-associated gene A
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CCK-2	Cholecystokinin receptor sub-type 2
cGMP	Cyclic guanosine monophosphate
CHS	College of Health Sciences
cNOS	Constitutive nitric oxide synthase
COX	Cyclooxygenase
CYP3A4	Cytochrome p450 3A4
CYP2D6	Cytochrome p450 2D6
ECL	Enterochromaffin-like
EGF	Epidermal growth factor
EP	E prostanoid receptors
GAE	Gallic acid equivalent
GI	Gastrointestinal
GIT	Gastrointestinal tract
GPx	Glutathione peroxidase
GSH	Glutathione
H+/K+-ATPase	Hydrogen-potassium-adenosine triphosphatase
H_2RAs	Histamine-2 receptor antagonists

HCl	Hydrochloric acid
HCO^-_3	Bicarbonate
H. pylori	*Helicobacter pylori*
HSP	Heat shock protein
IL-1β	Interleukin-1β
IL-6	Interleukin -6
ip	Intraperitoneal
IP	I prostanoid receptors
MDA	Malondialdehyde
ME	80% methanol extract
mEq/L	Milliequivalent per liter
NC	Negative control
NO	Nitric oxide
NOS	Nitric oxide synthase
NSAIDs	Nonsteroidal anti-inflammatory drugs
OECD	Organization for Economic Cooperation and Development
OMP	Omeprazole
P-CABs	Potassium-competitive acid blockers
PGs	Prostaglandins
PGE2	Prostaglandin E2
PGI2	Prostaglandin I2
PPIs	Proton pump inhibitors
PUD	Peptic ulcer disease
QE	Quercetin equivalent
ROS	Reactive oxygen species
S. abyssinica	*Stephania abyssinica*
SEM	Standard error of the mean

SOD	Superoxide dismutase
SOP	School of Pharmacy
SPSS	Statistical Package for Social Sciences
TFC	Total flavonoids content
TFFs	Trefoil factors
TGF-α	Transforming growth factor-alpha
TNF-α	Tumor necrosis factor-alpha
TPC	Total phenols content
UI	Ulcer index
UN	Number of ulcer per animal
UP	Percentage of animals with ulcers
US	Ulcer severity score per animal
UV	Ultraviolet
ZES	Zollinger-Ellison Syndrome

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1. Introduction

1.1. Overview of peptic ulcer disease

Peptic ulcer disease (PUD) is the most common gastrointestinal tract (GIT) disorder, affecting millions of people around the world (Singh *et al*., 2018), with an estimated incidence of 10-19% in the general population (Alsinnari *et al*., 2022). It is an acid-related GIT injury that usually affects the stomach and/or proximal duodenum and causes a mucosal breach that extends to the submucosa (Lanas and Chan, 2017). This occurs due to an imbalance between stomach acid-pepsin and mucosal defense barriers (Singh *et al*., 2018). Peptic ulcers are classified as gastric or duodenal ulcers based on the site of ulceration, and the symptoms vary depending on the type of ulcers and complications (Vimala and Gricilda Shoba, 2014). Abdominal pain, bloating, burning sensation, nausea, vomiting, anorexia, hematemesis, and melena are some of the clinical symptoms (Dunlap and Patterson, 2019), while bleeding, perforation, penetration, and gastric outlet obstruction are all life-threatening complications of PUD (Milosavljevic *et al*., 2011).

Several studies have found that there are several risk factors for the occurrence of peptic ulcers, including *Helicobacter pylori* (*H. pylori*) infections, prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), smoking, alcohol consumption, (Lee *et al*., 2017; Seo *et al*., 2019), and stress (Deding *et al*., 2016). Of these, the former two are the major risk factors involved in the pathogenesis of PUD (Fashner and Gitu, 2015; Hunt and Yuan, 2011).

1.2. Physiology of gastric acid secretion

Gastric acid regulates gastric function, defends against gastrointestinal (GI) pathogens, facilitates digestion and absorption of certain nutrients, and may influence feeding behavior (Pohl *et al*., 2008). Although neural and hormonal mediators have a role in gastric function, acid production is the stomach's unique and primary contribution to the digestive process (Ramsay and Carr, 2011). The epithelium of the stomach is made up of pits and glands. Two important functioning zones are the oxyntic gland area, representing about 80% of the organ, and the pyloric gland area, representing the remaining 20% (Mejia and Kraft, 2009). The principal cell in the gastric glands is the acid-secreting parietal cells (Jain *et al*., 2007).

Gastric acid secretion is regulated by neural (acetylcholine), hormonal (gastrin), and paracrine (histamine, somatostatin) mechanisms (Herszényi *et al*., 2019). In the neural pathway, acetylcholine (Ach) released by the vagal nerve stimulates gastric acid secretion directly by binding to muscarinic M_3 receptors on parietal cells and indirectly by stimulating the release of histamine from enterochromaffin-like (ECL) cells in the fundus and gastrin from G cells in the gastric antrum (Jain *et al*., 2007). Gastrin is released by antral G cells in response to luminal peptides and amino acids obtained from the diet. It stimulates gastric acid secretion by binding to gastrin or cholecystokinin-2 (CCK_2) receptor on parietal and ECL cells, and triggers ECL cells to secrete histamine (O'Connor and O'Moráin, 2014). Then, histamine produced from ECL cells binds to histamine 2 receptors and stimulates acid secretion in the parietal cells (Schubert and Peura, 2008).

The final step in acid secretion is the stimulation of proton pump or hydrogen-potassium-adenosine triphosphatase (H+, K+-ATPase) enzyme expressed in parietal cells, which mediates the exchange of cytoplasmic H^+ for luminal K^+. Then, H^+ secreted into the gastric lumen reacts with luminal Cl- to form hydrochloric acid (HCl), the main component of gastric acid (Mejia and Kraft, 2009; Ramsay and Carr, 2011). However, secretion of somatostatin from antral and corpus D cells comprises a negative-feedback loop, inhibition of gastric acid secretion (Joseph *et al*., 2003; O'Connor and O'Moráin, 2014).

Secretion of HCl in the stomach stimulates the release of pepsinogen from the peptic chief cells. The low pH allows pepsinogen to unfold and cleave itself autocatalytically, thereby generating pepsin, active form (O'Connor and O'Moráin, 2014). Pepsin's proteolytic activity on an average protein substrate decreases rapidly at pH >3 (Allen and Flemström, 2005; Bardhan *et al*., 2012).

1.3. Pathophysiology of peptic ulcer disease

Under normal circumstances, gastric acid secretion and gastroduodenal defense are in equilibrium. Injury to the mucosa occurs when the equilibrium between defensive and aggressive factors is disrupted, resulting in peptic ulcer(s) (Dunlap and Patterson, 2019).

1.3.1. Protective factors

The protective factors to maintain the structural and functional integrity of gastroduodenal mucosa from injurious substances include protective mucous barrier, formation of intracellular tight

junctions between gastric epithelial cells, cell renewal, increased gastric blood flow, and neutralization of reactive oxygen species as well as secretion of bicarbonates, prostaglandin and nitric oxide (deFoneska and Kaunitz, 2010).

The mucus-bicarbonate barrier is an important initial line of defensive factor against gastric acid and pepsin injury, and it has been demonstrated in all animals, including humans (Hogan *et al*., 1994). The GIT is protected by mucus, which coats the epithelial surface from the stomach to the colon (Rodríguez-Piñeiro *et al*., 2013). Mucus that protects GIT's epithelial surface is rich in mucins, which are highly glycosylated proteins with abundant serine and threonine-backbones linked to oligosaccharide side-chains that contribute to the formation of gel-like structure (Kim and Ho, 2010; Rodríguez-Piñeiro *et al*., 2013), and are synthesized and secreted by specialized epithelial mucus-producing cells called goblet cells (Rodríguez-Piñeiro *et al*., 2013). Different types of mucins are found in the GIT and include MUC2, MUC5AC, MUC6, and MUC5B. MUC5AC and MUC2, two gel-forming mucins, are the primary components of mucus in the stomach and intestine, respectively (Holmén Larsson *et al*., 2013; Pelaseyed *et al*., 2014).

The protective mucus layer varies greatly throughout the digestive tract. There is a two-layered mucus system in the stomach and colon, consisting of an inner, adherent and an outer, loose mucus layer, whereas the small intestine has a single-layered mucus system (Ermund *et al*., 2013; Johansson *et al*., 2013). This suggests a functional organization of the intestinal mucus system, with loose and penetrable mucus in the small intestine that may allow nutrients to pass through easily, as opposed to the stomach, where mucus provides physical protection, and the colon, where mucus separates bacteria from the epithelium (Ermund *et al*., 2013). The main function of the adherent mucus gel layer is a structural one to form a stable, unstirred layer that helps surface acid neutralization and acts as a physical barrier against luminal pepsin (Allen and Flemström, 2005; Phillipson *et al*., 2008).

The secretion of bicarbonate (HCO_3) into an adhering mucus gel layer maintains the pH gradient at the epithelial surface of the stomach and duodenum, and serves as the first line of defense against gastric acid (Allen and Flemström, 2005; Laine *et al*., 2008). As a result, mucus-bicarbonate barrier is the only system that keeps the epithelium from coming into contact with the stomach lumen. When this protective barrier is breached during pathologic conditions, other defense mechanisms are activated including fast epithelial cell renewal, intracellular acid neutralization, and mucosal

blood flow (Laine *et al*., 2008; Zatorski, 2017). Moreover, a continuous layer of gastric epithelial cells that secrete mucus and bicarbonate also produce heat shock proteins (HSPs), prostaglandins (PGs), trefoil factors (TFFs), cathelicidins, and defensins as the next line of mucosal defense (Tulassay and Herszényi, 2010).

Prostaglandins (PGs), which are produced by gastric epithelial cells from arachidonic acid with the help of the cyclooxygenases (COX) enzyme, are crucial in the protection of the gastric and duodenal mucosa against injury (Cryer, 2001). PGs, especially prostaglandin E2 (PGE2) and prostacyclin (PGI2), are potent vasodilators that have been shown to have cytoprotective effects on GI epithelium by maintaining gastric mucosal integrity and promoting ulcer healing. The ability of PGs to induce mucus-bicarbonate secretion and increase stomach blood flow may be responsible for their cytoprotective effect (Abdel-Salam *et al*., 2001; Dey *et al*., 2006). PGE2 exerts various physiologic activities in the GIT via E prostanoid (EP) receptors (EP1-EP4) (Dey *et al*., 2006), while that of PGI2 via I prostanoid (IP) receptor (Nishio *et al*., 2007). The mucosal defensive action of PGs is mainly mediated via receptors: EP1 enhances bicarbonate secretion (Takeuchi *et al*., 2006) and mucosal blood flow (Araki *et al*., 2000; Komoike *et al*., 2003); EP3 (Kato *et al*., 2005; Nishio *et al*., 2007) and IP (Nishio *et al*., 2007) inhibit acid secretion; and EP4 mediates mucus and bicarbonate secretion as well as facilitate ulcer healing (Aoi *et al*., 2004; Larsen *et al*., 2005). Altogether, almost the entire mucosal defense mechanisms are stimulated by prostaglandins. They inhibit acid secretion, enhance mucosal blood flow, stimulate mucus and bicarbonate secretion, and accelerate epithelial cell regeneration and mucosal ulcer healing (Laine *et al*., 2008).

HSPs are also generated by gastric epithelial cells in response to various stresses, including oxidative stress, heat, chemicals, and cytotoxic agents (Hirata *et al*., 2009; Tanaka *et al*., 2007). They prevent protein denaturation and protect cells from harmful effects of stress due to their role as ‘molecular chaperones’ (Tóth *et al*., 2015). HSPs, specifically HSP70, have been linked to gastroprotection and ulcer healing (Sidahmed *et al*., 2013), most probably by protecting key cytoprotective enzymes (COX and nitric oxide synthase (NOS)) (Choi *et al*., 2009; Tsukimi and Okabe, 2001).

TFF peptides are important secretory products of mucous epithelia that assist the preservation of mucosal integrity by stabilizing the mucus barrier and promoting restoration processes (Hernandez

et al., 2009). Other peptides produced by the gastrointestinal epithelium include defensins and cathelicidins, which are antimicrobial peptides that prevent bacterial colonization and promote ulcer healing by increasing cell proliferation and angiogenesis (Wehkamp *et al*., 2007; Yang *et al*., 2006).

The other important protective factor is epithelial cells renewal, which are in a constant state of renewal in order to keep the stomach mucosa intact (Alison and Sarraf, 1994). Progenitor cells proliferate in a well-coordinated and controlled manner in the epithelium, allowing injured or old surface epithelial cells to be replaced. The gastrointestinal surface epithelium usually regenerates in 3–7 days, whereas glandular cells regenerate in months (Laine *et al*., 2008). By activating their common receptor, transforming growth factor-alpha (TGF-α) and epidermal growth factor (EGF) regulate cell proliferation in the gastric mucosa. TGF-α is primarily involved in normal conditions and after an acute injury, whereas EGF is predominantly involved during the healing of chronic ulcers (Jones *et al*., 1999).

Gastric mucosal blood flow included under protective factors maintains the normal gastric mucosa and heals the damaged mucosa by supplying oxygen, nutrients, and bicarbonate to the surface epithelial cells (Sørbye and Svanes, 1994). In addition, blood circulating through the surface mucosa eliminates waste or toxic materials and prevents hydrogen ion back diffusion (Kawano and Tsuji, 2000; Sørbye and Svanes, 1994). Moreover, nitric oxide (NO) synthesized by constitutive nitric oxide synthase (cNOS) mediates multiple physiological functions in the GIT, such as maintenance of GI mucosal integrity, maintenance of vascular tone, and gastric blood flow (Lanas, 2008). It also stimulates gastric mucus secretion and decreases histamine-stimulated acid secretion by raising cyclic guanosine monophosphate (cGMP) levels in the parietal cells involving the activation of guanylate cyclase (Cho, 2001; Lanas, 2008; Stanek *et al*., 2008). Additionally, NO is involved in the healing process of chronic gastric ulcers by enhancing blood flow to the ulcer site and increasing the number of capillaries in the granulation tissue at the ulcer margin (Stanek *et al*., 2008).

1.3.2. Aggressive factors

Peptic ulcers are caused by a number of factors. Then, continuous exposure of gastric mucosa to that of potentially harmful agents such as gastric acid-pepsin, bile acids, *H. pylori* infection, drugs

like NSAIDs, and food ingredients has been linked to the pathophysiology of PUD (Magaji *et al*., 2008; Raju *et al*., 2009).

Despite its role in digestion and pathogen defense, gastric acid remains an important causative factor for a variety of common upper GI disorders (Schubert and Peura, 2008). However, the first step toward ulcer formation is a defect in the defensive mechanism of the gastric mucosa (Gundamaraju *et al*., 2014). There are a few uncommon conditions that cause abnormally high gastric acid secretion and ulcers, such as chronic hypercalcemia, decreased somatostatin secretion, hypergastrinemia, and Zollinger-Ellison syndrome (ZES) (Schubert and Peura, 2008). For instance, the distribution of gastric *H. pylori* infection may cause ulcerations in the duodenum and stomach by increasing gastrin secretion (Waldum *et al*., 2014). Hence, persistent hypergastrinemia causes parietal cell proliferation and increased gastric acid production, which leads to ulcer formation, notably in the duodenum (Zatorski, 2017).

In contrast to acid, pepsin, the other endogenous aggressor in gastric juice, has received comparatively less attention. Because luminal pepsin cannot traverse the continuous adhering mucus layer in a physiologically meaningful time due to its relatively large molecular size. Nonetheless, it slowly hydrolyzes and erodes the mucus layer at the acidic pH, but a new secretion counteracts mucus loss at the same time (Allen and Flemström, 2005). Unfortunately, pepsin-related mucosal injury and its role in peptic ulcers are not clearly understood and need further studies (Zatorski, 2017).

It is well-known that NSAIDs are common factors in the pathophysiology of PUD. The key mechanism by which NSAIDs cause peptic ulcers and PUD related complications is through inhibition of the COX, which is required for the synthesis of PGs (Melcarne *et al*., 2016; Narayanan *et al*., 2018). COX has two well-known isoforms, COX-1 constitutively found in most cells and COX-2 induced at sites of inflammation (Coruzzi *et al*., 2007; Drini, 2017). COX-1-derived PGs maintain stomach mucosal integrity by regulating mucosal blood flow and the secretion of epithelial cell mucus and bicarbonate, whereas COX-2-derived PGs influence epithelial cell proliferation and endothelial-leukocyte adherence at the ulcer site (Konturek *et al*., 2005). According to Wallace *et al*. (2000), inhibition of both isoforms may play a role in the pathogenesis of NSAID-induced gastric damage via different mechanisms. Inhibiting COX-1

reduces gastric blood flow, and inhibiting COX-2 increases leukocyte adherence to the vascular endothelium, resulting in gastrointestinal ulceration.

The other mechanism of NSAIDs induced gastric ulcers is through topical effects. Aspirin and other NSAIDs (indomethacin, naproxen, ibuprofen and diclofenac…etc.) are weak non-ionized acids that can readily penetrate the mucus layer and enter epithelial cells (Lauret *et al*., 2015). In the epithelial cells (pH 7.4), they ionize and release H+, unable to cross the lipid membrane and become trapped. This leads to uncoupling of oxidative phosphorylation, resulting in reduced mitochondrial energy production, increased cellular permeability, and decreased cellular integrity (Narayanan *et al*., 2018). Permeability and formation of unstable pores can facilitate back-diffusion of luminal acid (Lichtenberger *et al*., 2006). This can result in a topical damage and fast epithelial cell death, superficial erosions, and hemorrhage (Narayanan *et al*., 2018). Therefore, the physicochemical properties of NSAIDs appear to be important in topical irritation and short-term stomach injury (Bjarnason *et al*., 2007).

H. pylori, Gram-negative bacillus bacterium, plays a vital role in the pathophysiology of PUD as well. Motility, adhesion, and acid acclimation are among the mechanisms that allow this bacterium to colonize and survive in a harsh acidic environment of the stomach. The flagellar system allows bacteria to move through the gastric mucus layer in search of the optimal conditions for survival and aids its attachment to the epithelial surface of the mucosa (Marcus and Scot, 2016). Its adherence to the gastric epithelium is necessary for establishing infection because it protects the bacteria from clearance mechanisms such as gastric peristalsis, bulk flow of gastric fluid, and the constant shedding and replenishment of the mucus layer (Marcus and Scot, 2016; Oleastro and Ménard, 2013). It also facilitates evasion from the human immune system and efficient delivery of proteins like cytotoxin-associated gene A (CagA) oncoprotein into gastric cell. As a result, bacteria with better adhesion properties infiltrate the host at a higher density (Oleastro and Ménard, 2013). Furthermore, urease activity is the cornerstone of acid acclimation, which allows *H. pylori* to colonize the stomach's acidic environment (Rutherford, 2014). Urease is a urea amidohydrolase that catalyzes the hydrolysis of urea to produce ammonia (NH_3) and carbon dioxide (CO_2), raising the periplasmic pH and facilitating *H. pylori* survival (Magaji *et al*., 2008; Miller and Maier, 2014). Aside from raising pH, urease-generated ammonia is toxic to gastric epithelial cells because of its adherence properties (Mégraud *et al*., 1992). All in all, the bacterial adaptation mechanism to the

negative effects of acidic pH is a complex mechanism involving bacterial factors such as flagella, shape, enzymes, and proteins as well as environmental factors; mucus, urea, and acid (Ansari and Yamaoka, 2017).

Another factor in the pathophysiology of PUD is alcohol consumption, which disrupts the gastric mucosal barrier and enhances the mucosal permeability. The changes induced by short-term exposure to alcoholic beverages are rapidly reversible, whereas prolonged alcohol consumption can impair microcirculation and advance structural mucosal injury (Zatorski, 2017). In addition, consumption of ethanol may activate the innate immune system, causing the release of pro-inflammatory cytokines like interleukins (IL-6, IL-1β), and tumor necrosis factor-α (TNF-α). An increased levels of these cytokines enhance gastric mucosal inflammation, vascular congestion, and injury (Chang *et al*., 2015). Thus, ethanol-induced gastric ulcers are associated with an accumulation of inflammatory cells and a high production of reactive oxygen species (ROS), which can lead to gastric oxidative damage (Nordin *et al*., 2014; Zhou *et al*., 2020).

Oxidative stress is the disturbance of oxidant–antioxidant homeostasis and plays a role in a variety of diseases, including peptic ulcers and gastric carcinoma (Tandon *et al*., 2004). It is characterized as an elevated level of ROS and causes a number of conditions that promote either additional ROS production or a reduction in antioxidant defenses (Suzuki *et al*., 2011). ROS, such as hydrogen peroxide, hydroxyl radicals, and superoxide anions are involved in the pathogenesis of various diseases, while antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are involved in protecting cells from the damaging effects of ROS. At the physiologic level, ROS maintains GI homeostasis by killing invading pathogens, tissue repair process, and wound healing. However, continuous stimulation of the GI mucosa by exogenous agents (NSAIDs, infection, alcohol, cigarette smoking and ultraviolet radiation) can result in prolonged inflammation and excessive production of ROS, disrupting bodily homeostasis and causing oxidative tissue damage (Bhattacharyya *et al*., 2014).

Caffeinated drinks such as tea and coffee are also involved in the pathophysiology of PUD. Their mechanism in the pathogenesis of PUD may be by stimulating gastric acid secretion and decreasing PGE2 synthesis (Gulia and Choudhary, 2011). In addition, Kadhim *et al*. (2015) report showed that there is a positive relation between coffee consumption and *H. pylori* infection.

Accordingly, those individuals who drink coffee are positive for *H. pylori* infection than those who do not drink coffee, but the mechanisms related to this association need further studies.

1.4. Treatment of peptic ulcer disease

Peptic ulcer treatment aims to alleviate ulcer pain, improve ulcer healing, reduce ulcer-related complications, and prevent ulcer recurrence (Gulia and Choudhary, 2011). Treatments are classified as non-pharmacological and pharmacological.

Non-pharmacological therapies

Non-pharmacological therapies for PUD include avoiding alcohol and caffeine, discontinuing or reducing NSAIDs usage, reducing psychological stress, quitting smoking, and avoiding foods and beverages that aggravate ulcer symptoms (Gulia and Choudhary, 2011).

Pharmacological therapies

The therapeutic algorithms for the treatment of peptic ulcers differ based on ulcer history or complications (Kamada *et al*., 2021). Fundamental pharmacologic PUD treatment paradigm shifts from acid neutralization to acid suppression and later the recognition of the role of *H. pylori* infection (Manu *et al*., 2021). They may be classified based on the mechanism of action as: antacids, mucosal protective, and antisecretory drugs. For instance, antacids are a group of alkaline compounds used for the neutralization of excessive gastric acid in the stomach (Sałaga and Mosińska, 2017). Sucralfate is a cytoprotective drug that forms a protective barrier between epithelium and damaging agents. It also enhances the local levels of fibroblast growth factors and induces a rise in the mucosal concentration of PGs (Candelli *et al*., 2000). Moreover, misoprostol is a PGE1 analog that possess cytoprotective properties by stimulating bicarbonate and mucus secretion, as well as the prevention of epithelial cell disruption and improved mucosal blood flow (Sałaga and Mosińska, 2017). Along with this, it reduces acid secretion by binding to a PG receptor on parietal cells and lowering histamine-stimulated cyclic adenosine monophosphate (cAMP) generation (Davies *et al*., 2001; Gulia and Choudhary, 2011).

Another major class of medication in the management of PUD are anti-secretory drugs that include anticholinergics, proton pump inhibitors (PPIs), and histamine-2 receptor antagonists (H_2RAs). For example, pirenzepine is a selective anticholinergic agent that decreases acid secretion by blocking muscarinic (M1) receptors for acetylcholine and effectively inhibits vagally stimulated

acid secretion. But it is rarely used due to its poor efficacy, substantial and unwanted anticholinergic side effects, and risk of blood problems (Gulia and Choudhary, 2011). PPIs, such as omeprazole, pantoprazole, esomeprazole, rabeprazole, lansoprazole, and dexlansoprazole are widely used in the treatment of acid-related GI problems (Scarpignato *et al*., 2016; Strand *et al*., 2017). Their action is mediated through irreversible inhibition of H+/K+-ATPase, the final step in acid production, in the gastric parietal cells (Alfahad *et al*., 2021; Bruno *et al*., 2019). In addition, H_2RAs (cimetidine, ranitidine, famotidine, and nizatidine) block the H_2R in the gastric parietal cells, resulting in less acid and pepsin production (Van der Pol *et al*., 2014). They mostly reduce basal secretion, which is activated by histamine, but they also have a little influence on meal-induced acid production, which is strongly stimulated by gastrin and acetylcholine (Brinkworth *et al*., 2016).

In all patients with *H. pylori*-related PUD, eradication of *H. pylori* is recommended. First-line therapy usually initiated with a PPI and two antibiotics such as, amoxicillin, and clarithromycin or metronidazole for 7-14 days (Fashner and Gitu, 2015; Feng *et al*., 2016). In areas of high clarithromycin and/or metronidazole resistance, bismuth-based quadruple therapy (PPI, bismuth salicylate, metronidazole, and tetracycline) or non-bismuth concomitant quadruple should be recommended as first line treatment (Suzuki *et al*., 2019). A fluoroquinolone-based triple therapy should be used if bismuth-containing quadruple therapy fails (Gisbert, 2020; Selgrad and Malfertheiner, 2017).

Moreover, several new PPIs including ilaprazole, azeloprazole, and anaprazole (Hunt and Scarpignato, 2018), and novel potassium-competitive acid blockers (P-CABs) such as vonoprazan and tegoprazan, revaprazan are developed for the treatment of PUD (Scarpignato and Hunt, 2021; Wattana and Tawithep, 2022).

1.5. Medicinal plants used for the management of peptic ulcer disease

Medicinal plants have been used in folklores around the world for the treatment of various diseases since prehistoric times (Awuchi, 2019). In developing countries including Ethiopia, around 80% of populations use plant-based drugs for their health care needs (Hishe *et al*., 2016). This could be due to their easy availability and affordability compared to modern medicines (Ahmed *et al*., 2021).

Plant derived medicines have been used in Ethiopia to treat a number of illnesses, including peptic ulcer diseases. A wide variety of plants from various families are employed as antiulcer medicinal plants by harvesting different plant parts, with leaves being the most commonly used (Tadesse *et al*., 2022).

In the scientific literature, a large number of medicinal plants with antiulcer activities have been reported. Among them, *Ficus thonningii* (Adane *et al*., 2021), *Croton macrostachyus Hocsht* (Mekonnen *et al*., 2020), *Osyris quadripartita Decne* (Gelayee *et al*., 2017), *Rumex nepalensis* (Zewdu and Aragaw, 2020), *Cordia africana Lam* (Yismaw *et al*., 2020), *Plantago lanceolata* (Melese *et al*., 2011), *Solanum incanum L* (Belayneh *et al*., 2021), *Calpurnia aurea* (Ait.) Benth. (Andargie *et al*., 2022), and *Urtica simensis* (Ahmed *et al*., 2022) are some of the medicinal plants that have been reported with significant antiulcer activity against various ulcer models in Ethiopia.

1.6. Overview of the experimental plant

Stephania abyssinica (Quart. -Dill. & A. Rich.) Walp. is a medicinal plant belonging to the family Menispermaceae containing around 65 genera and 350 species of plants (Semwal *et al*., 2010). *Stephania abyssinica* (*S. abyssinica*) has two varieties: *S. abyssinica var. abyssinica* with glabrous sepals and *S. abyssinica var. tomentella* with densely pubescent to tomentose sepals. *S. abyssinica var. abyssinica* (Figure 1) is found throughout tropical Africa, including Ethiopia, and grows on the ground or over shrubs in full sunlight and high humidity, primarily on the edges of forests and distributed areas adjacent to roads (De Wet *et al*., 2014).

S. abyssinica is a climbing shrub or twining liana, woody at base, stem covered with thin bark, prominent longitudinal ridges, and branchlets glabrous. The leaves are ovate to broadly oval, rounded at the base, obtuse or subacute at the apex, membranous, glabrous, with 8–10 basal nerves and a 4–12 cm long petiole (Bussmann *et al*., 2021). The flowers are cream or reddish in color. It flowers from spring to early autumn (De Wet *et al*., 2014).

Most of plants belonging to genus *Stephania* have been used as traditional medicines to treat dyspepsia, diarrhea, tuberculosis, sore-breasts, dysmenorrhea, urinary disorders, ascariasis, abdominal pain, indigestion, asthma, leprosy, wounds, and headaches for centuries (Semwal *et al*., 2010). In African countries, different parts of *S. abyssinica* are thought to be an effective medicinal remedy. In Nigeria, the leaves of *S. abyssinica* have been used to treat menstrual disorders and

childbirth issues (Kadiri *et al*., 2015). In Kenya, it has been used as anti-malarial therapy by the communities living in the South Nyanza (Omole *et al*., 2014).

In Ethiopia, it is known by the vernacular names '*Etse eyesus*' or '*ye ayit hareg'* in Amharic and '*Kalaalaa*' in Afan Oromo, and it is widely utilized in traditional medicine. The leaves of the plant have been used for the treatment of various disorders including rabies (Giday *et al*., 2010), abdominal pain and *kuruba* (Birhanu *et al*., 2015). In addition, fresh leaves decocted with water are used to treat gastritis (Duressa, 2016; Suleman and Alemu, 2012) and snake bites (Suleman and Alemu, 2012). The juice of leaves mixed with butter is given for babies' sickness, and the juice of leaf and stem is also taken orally for stomachache and headache (Teklehaymanot and Giday, 2007). The roots are also used for the management of wound (Giday *et al*., 2007), stomachache, and retained placenta (Giday *et al*., 2009), and the whole part of plant is used to treat common cold (Megersa *et al*., 2013).

The genus *Stephania* is well known by its alkaloidal compounds. Regarding this, various alkaloidal compounds of genus *Stephania* have been isolated and revealed promising pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antitumor, antiviral, antinociceptive, cardioprotective, neuroprotective, and anti-multidrug resistance (Wang *et al*., 2022). For instance, two bisbenzylisoquinoline alkaloids and one hasubanane alkaloid were isolated from *S. abyssinica* leaf extract (Omole *et al*., 2014).

Some traditional uses of different parts of *S. abyssinica* have been supported scientifically for its medicinal activities by using several pharmacological studies, including hepatoprotective (Washe and Fanta, 2016), antimalarial (Zemene *et al*., 2020), antihypertensive (Fodem *et al*., 2021), analgesic and anti-inflammatory (Leyikun, 2015), antidiarrheal and antispasmodic (Tatek, 2010), antineoplastic (Abebe, 2016), antiviral (Asres et al., 2001), wound healing (Yiblet *et al*., 2022), repellent activity against malaria vector *Anopheles arabiensis* (Jemberie *et al*., 2016), and nutritional value (Feudjio *et al*., 2020).

Despite the claims of *S. abyssinica* leaves to treat gastritis, there is no report in the literature regarding its gastroprotective activity. As a result, the aim of this study is to evaluate its gastroprotective activity.

Figure 1: Photograph of *Stephania abyssinica* (photo captured by Banchayehu Firehun, July 22, 2022)

1.7. Rationale for the study

Peptic ulcer disease has become a common global health problem that affects nearly 10% of the world population with different etiologies (Sahoo *et al*., 2016). The prevalence, recurring characteristics, and potentially serious complications of PUD cause significant morbidity, mortality, and economic loss (Xie *et al*., 2022; Yismaw *et al*., 2020).

Despite the recent decrease in the incidence and rate of hospital admission for uncomplicated PUD in developed countries, hospitalization for peptic ulcer complications such as bleeding and perforation has risen considerably among advanced age groups (Roy *et al*., 2013). These GI complications and their recurrence can be challenging to treat and frequently necessitate surgical intervention (Napolitano, 2009). According to Rickard. (2016) report, the most common reason for peptic ulcer related surgery in Sub-Saharan Africa was perforation (35%), followed by

obstruction (30%), chronic disease (28%), and bleeding (7%). In Ethiopia, a retrospective study of patients operated at the Minilik II Memorial Referral Hospital for acute perforated peptic ulcers showed that the majority of patients had operations for perforation of the anterior part of the duodenum (Bekele *et al*., 2017).

Conventional drugs such as H_2RAs, PPIs, prostaglandins analogs, antacids and antimicrobials are available for the management of PUD, but they have various adverse effects (Gupta *et al*., 2021). For instance, long-term use of PPIs may cause gastric carcinoids, bone fractures, hypomagnesemia, nutritional deficiencies (vitamin B12, calcium, and iron), and enteric infections, most notably *Clostridium difficile* (Fossmark *et al*., 2019; Savarino *et al*., 2016). Whereas H_2RAs (cimetidine) use can cause impotence, and gynecomastia as well as medication interactions related to cytochrome P450 inhibition, particularly CYP3A4 and 2D6 (Mejia and Kraft, 2009).

The other major issue with the conventional drugs is ulcer recurrence. Despite a healing rate of 80-100% after 4-8 weeks of therapy with PPIs and H_2RAs, ulcer recurrence rate is 40-80% within one year after discontinuing the therapy (Kommu *et al*., 2013). Moreover, antibiotic resistance in *H. pylori* has reached alarming levels worldwide, having a significant impact on treatment efficacy and being associated with an increased risk of treatment failure (Matsumoto *et al*., 2019; Savoldi et al., 2018).

Overall, the impact of PUD on health-care systems is enormous, emphasizing the need of safe, effective, and affordable gastroprotective agents (Alsinnari *et al*., 2022; Kuna *et al*., 2019). Herbal medicines are becoming a viable alternative treatment to available conventional drugs for the management of peptic ulcers, possibly due to their lower cost, perceived effectiveness, and availability, as well as the fact that they have few or no adverse effects (Akinwumi and Sonibare, 2019; Sharifi-Rad *et al*., 2018). A wide variety of medicinal plants have been used in traditional medicines to treat peptic ulcers, *S. abyssinica* is one of them. Therefore, this study aimed to evaluate the gastroprotective activity of *S. abyssinica* in experimentally induced gastric ulcer models and confirm its traditional use as an anti-gastritis.

2. Objectives

2.1. General objective

- To evaluate the gastroprotective activities of aqueous and 80% methanol leaf extracts of *Stephania abyssinica* in rats

2.2. Specific objectives

- To determine the acute oral toxicity of aqueous and 80% methanol leaf extracts of *S. abyssinica*;
- To determine gastroprotective activities of aqueous and 80% methanol leaf extracts of *S. abyssinica* against pyloric ligation induced gastric ulcer in rats;
- To assess gastroprotective activities of aqueous and 80% methanol leaf extracts of *S. abyssinica* against indomethacin induced gastric ulcer in rats;
- To evaluate gastroprotective activities of aqueous and 80% methanol leaf extracts of *S. abyssinica* against ethanol induced gastric ulcer in rats;
- To perform phytochemical screening of aqueous and 80% methanol leaf extracts of *S. abyssinica*;
- To quantify the phytochemical constituents of aqueous and 80% methanol leaf extracts of *S. abyssinica*.

3. Materials and methods

3.1. Drugs, chemicals, and reagents

Indomethacin (Leben laboratories pvt. ltd, India), Omeprazole (Kopran Limited, India), Misoprostol (Naari Pharma Private Limited, India), Ketamine Hydrochloride USP (Neon Laboratories Limited, India), Diazepam (Cenexi sas Fontenay-sous-Bois, France), distilled water (Department of Pharmaceutics and Social Pharmacy, Addis Ababa University (AAU) and Kilitch estro biotech PLC, Oromia, Ethiopia), Methanol (Blulux laboratories, India), Sodium hydroxide (Ranchem industry, Turkey), Phenolphthalein (Ranchem industry and trading, India), Methyl orange (Dalul Pharmaceuticals PLC, Ethiopia), Alcian blue 8GX AR (Ozone international, India), Sucrose (Labchemical, India), Sodium acetate (Abron chemicals, India), Magnesium chloride, Diethyl ether, Glacial acetic acid, Sodium nitrite, Aluminum chloride hexahydrate, Chloroform, and Tween 80 (Loba chemie Pvt. Ltd., India), Ferric chloride hexahydrate (Techno Pharmchem Bahadurgarh, India), Sulphuric acid, Atropine, and Bromocresol green (BDH chemicals LTD, England), Hydrochloric acid (DPP laboratory reagent, Ethiopia), Quercetin dihydrate (Sigma Aldrich, Germany), Gallic acid and Follin Ciocalteu (Merck, Germany), Sodium carbonate (Neolab, India) and Ethanol (Favor Trading PLC, Ethiopia) were used in the study. All drugs were purchased from pharmacies in Ethiopia, and most chemicals and reagents were also purchased from their respective suppliers in Ethiopia, while Alcian blue 8GX AR (Ozone international) was purchased from India. All were of analytical grades.

3.2. Experimental animals

Healthy Sprague Dawley rats (150–250 g, either sex) were used in the experiment. They were obtained from Department of Pharmacology and Clinical Pharmacy, School of Pharmacy (SOP), College of Health Sciences (CHS), AAU. All of them were kept in cages at room temperature with a 12/12 h light/dark cycle and given access to pellet food and water ad libitum. Prior to experiments, they were acclimatized to the laboratory condition for seven days. Moreover, animal handling and care were carried out in accordance with the internationally accepted laboratory animal care and use guidelines (National Research Council, 2010) and approved by the Ethics Review Committee of the SOP, CHS, AAU (ERB/SOP/462/14/2022).

3.3. Plant material

The leaves of *S. abyssinica* were collected from Ashewa Meda, Burayu, Sheger city administration, Ethiopia, on July 22, 2022. The plant specimen was recognized and authenticated by a taxonomist Mr. Melaku Wondafrash, and a voucher specimen (BF001) was deposited at the National Herbarium, College of Natural and Computational Sciences, AAU for future reference.

3.4. Plant extraction

Fresh leaves of *S. abyssinica* were thoroughly cleaned using water to remove dirt and then allowed to dry under shade. The dried leaves of plant were coarsely powdered with mortar and pestle. Then, this was subjected for aqueous and 80% methanol extraction.

3.4.1 Preparation of aqueous extract

Two hundred grams (200 g) of coarsely powdered leaves of *S. abyssinica* were boiled with distilled water (1:10 w/v) for 30 minutes, and then allowed to cool (Kaneria *et al*., 2012). After cooling, the mixture was filtered using a nylon cloth, and the filtrate was then further filtered by Whatman No. 1 filter paper using a pressurized suction filtering system (vacuum pump). The procedure was repeated to extract the plant material exhaustively. Filtrates from each procedure were collected, placed in a deep freezer (-20 ºC), and dried in a lyophilizer (Alpha 2-4 LD plus, Germany). Finally, the yield of the aqueous extract (AQ) was 29.5 g with a percentage yield of 14.75%, and it was then stored in a refrigerator until use.

3.4.2. Preparation of 80% methanol extract

The coarsely powdered leaves of the plant (300 g) were subjected to maceration with 80% methanol (1:10 (w/v)) for three days with intermittent agitation and stirring (Zemene *et al*., 2020). The solution was filtered with a nylon cloth followed by sterile filter paper (Whatman No.1) and the residue was re-macerated twice using the same procedure. Then, the resulting filtrates were combined and concentrated in a rotary evaporator (Heidolph Laborota 4001, Germany) at 40 °C, and the remaining extract was placed in the oven at 45 °C until dried. A total of 68g (22.67%) of methanol extract (ME) was obtained, preserved in a tightly closed vial and stored in the refrigerator until use.

3.5. Acute toxicity study

Acute oral toxicity test for both extracts was conducted according to Organization for Economic Cooperation and Development (OECD) guideline 425 (OECD, 2008). The study utilized ten female rats, five for each extract. After the period of fasting, two rats each received 2 g/kg limit test dose of an aqueous and 80% methanol extract, respectively. They were closely observed for any physical or behavioral changes during the initial thirty minutes, with extra focus during the first 4 h. The observation was continued for twenty-four hours, and no deaths were noted. As a result, the remaining eight rats (four for each extract) received the same dosage and were continuously monitored for behavioral, neurological, and physical abnormalities as well as mortality for the initial 4 h of the first day and then daily for the total of 14 days.

3.6. Grouping and dosing of animals

Forty-eight rats (either sex, weighing 150-250g) were used for each model and grouped into 8, each with 6 animals. Group I assigned as negative control (NC) received 10 ml/kg of 2% Tween 80 and group II assigned as positive control was treated with standard drugs: omeprazole (20 mg/kg) as anti-secretory agent for pylorus ligation induced gastric ulcer model and misoprostol (100 µg/kg) as cytoprotective agent for indomethacin and ethanol induced gastric ulcer models. Groups (III-V & VI-VIII) were treated with AQ and ME extracts of *S. abyssinica* at different doses of 100, 200, and 400 mg/kg, respectively. The extracts and standard drugs were dissolved in 10 ml/kg of 2% tween 80. The doses for both extracts were chosen based on the results of an acute oral toxicity study as per OECD guideline, with 10% of the limit test dose serving as the medium dose. Additionally, the doses of indomethacin (40 mg/kg) and ethanol (5 ml/kg) used to induce gastric ulcers, as well as standard drugs used as positive controls (omeprazole and misoprostol) were based on scientific literature and supported by pilot study prior to the actual experiment. Administration was performed orally via oral gavage.

3.7. Gastric ulcer induction

3.7.1. Pylorus ligation induced gastric ulcer

Gastric ulcer induction by pylorus ligation was performed using the shay method reported by Adane *et al*. (2021). Animals were fasted for 24 hours while having free access to water, and treated as mentioned under section 3.6. After 1 h of administration (vehicle/standard/extracts), animals were anesthetized with ketamine (50 mg/kg) and diazepam (5 mg/kg) ip and the abdomen

was opened by a small midline incision below the xiphoid process. The pyloric part of the stomach was carefully lifted out and ligated to avoid traction to the pylorus or injury to its blood supply. The stomach was replaced with care, and the wall was closed by interrupted sutures using silk and catgut. Following 4 h of pylorus ligation, the rats were sacrificed with an excess anesthetic agent (ketamine). The abdomen was opened, cardiac end of each stomach was ligated to prevent gastric contents loss, the stomach was then dissected, opened, and the contents were emptied into a centrifuge tube. Then, centrifuged and evaluated for total volume, pH, free, and total acidity. The stomach mucosa of each animal was washed with distilled water, labeled, and evaluated for ulcers. Ulcer index, % ulcer inhibition, mucin content, and % increment of mucin were calculated accordingly.

3.7.2. Indomethacin induced gastric ulcer

Indomethacin-induced gastric ulcer in rats was performed according to Ahmed *et al*. (2022). Rats were fasted for 24 hours prior to the experiment with free access to water and treated as mentioned under grouping and dosing of animals. After 1 h of treatment, 40 mg/kg of indomethacin was administered to rats to induce gastric ulcer. After six hours, animals were sacrificed by overdose of ketamine, and their stomachs were excised and opened along the greater curvature. After that, the stomachs were washed and evaluated for lesions as described above.

3.7.3. Ethanol induced gastric ulcer

Absolute ethanol (1 ml/200 g) was administered to induce gastric ulcer in rats after a 24 h fast (Sistani Karampour *et al*., 2019). Animals received vehicle, standard drug (misoprostol), or extracts one hour before ulcer induction, as described in section 3.6. They were sacrificed by overdose of ketamine after 60 minutes of ethanol administration, and their stomachs were dissected and opened along the larger curvature and bathed with distilled water to avoid remaining contents on the ulcerated region. Gastric ulceration was assessed by counting ulcers, calculating ulcer index, and determining mucin content as stated above for both models.

3.8. Evaluation of gastroprotective activity

3.8.1. Macroscopic evaluation of stomach

The stomach was opened along the greater curvature and washed using distilled water to remove any stomach contents including blood clots. It was then placed on a corkboard to assess the

formation of ulcers in the glandular area of the stomach and photographed with a phone camera for pictorial support. The number of ulcers per stomach was counted and the lesions were measured with a ruler. The severity of the ulcers was scored using the following scale (0 to 5) (Bhattamisra *et al*., 2019): almost no ulcers (0), mucosal edema and petechiae (1), 1-5 small ulcers (1-2 mm) (2), > 5 small or intermediate ulcers (3-4 mm) (3), ≥ 2 intermediate or one gross ulcers (> 4 mm) (4), perforation (5). Ulcer index and percentage ulcer inhibition were computed using the formula below (Zewdu and Aragaw, 2020).

$$UI = (UN+US+UP)/10$$

Where, UI= Ulcer index, UN = number of ulcers per animal, US = ulcer severity score per animal, UP = percentage of animals with ulcers.

$$\% \text{ ulcer inhibition} = \frac{UI\ control - UI\ test}{UI\ control} \times 100$$

3.8.2. Determination of gastric juice volume and pH

Gastric juice from each animal stomach was collected and centrifuged at 1000 rpm for ten minutes. After centrifugation, the supernatant was decanted, and the gastric juice volume and pH were measured with a measuring cylinder and pH meter (370 pH meter Janway, England), respectively (Gundamaraju *et al*., 2014). Then, gastric juice was subjected to free and total acidity determinations.

3.8.3. Determination of free and total acidity

Free and total acidity were determined by titrations with 0.01N sodium hydroxide (NaOH) using methyl orange and phenolphthalein as indicators, respectively (Zakaria *et al*., 2015). An aliquot of gastric juice (1ml) was pipetted into a beaker and diluted with 1 ml of distilled water. Then, 2 drops of methyl orange were added and titrated with NaOH until a yellowish orange color noticed. The volume of NaOH added was recorded, which corresponds to free acidity. Similarly, total acidity was determined as well. Two to three drops of phenolphthalein indicator were added, and the mixture was titrated with NaOH until a permanent pink color appeared. The volume of NaOH required was noted, which corresponds to total acidity. Acidity was calculated as mEq/L using the following formula:

$$\text{Acidity} = \frac{Volume\ of\ NaOH\ X\ Normality\ of\ NaOH\ X\ 100}{0.1}\ \text{mEq/L}$$

3.8.4. Estimation of gastric mucus content

The gastric mucus content was estimated by Corne et al. method described by Mekonnen *et al*. (2020). After ulcer scoring, the glandular segment of the stomach was removed, weighed, and immersed immediately in 10 ml of 0.1% Alcian blue (prepared in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate at pH of 5.8) for two hours. An excess dye was then removed by two subsequent rinses in 10 ml of 0.25 M sucrose solution at 15 and 45 minutes. The remaining dye-gastric mucus complex was extracted with 10 ml of 0.5 M $MgCl_2$ for two hours with occasional shaking (30-minute intervals). Five milliliters (5 ml) blue extract was mixed and shaken with an equal volume of diethyl ether (5ml). The resultant emulsion was then centrifuged at 3000 rpm for 15 minutes, and the absorbance of the aqueous layer was measured at 580 nm using a UV-visible spectrophotometer (Unico UV-2100, USA). The amount of Alcian blue extracted per gram of glandular tissue was determined using the standard curve, which was generated by taking different concentrations of Alcian blue (0.5 1, 2, 3, 4, and 5 µg/ml) (Figure 2), and mucin content was calculated by the following formula (Ahmed *et al*., 2022).

$$\text{Mucin content} = \frac{Alcian\ Blue\ (\frac{\mu g}{ml})}{glandular\ tissue(g)}$$

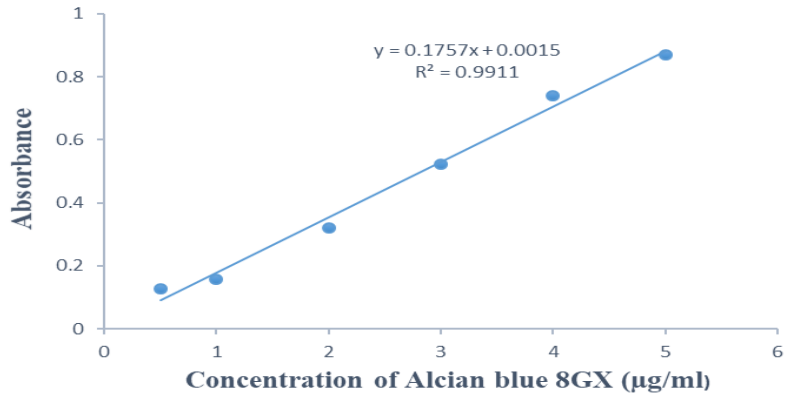

Figure 2: Calibration curve of Alcian blue 8GX

3.9. Phytochemical screening

Standard methods were used to determine the major secondary metabolites such as saponins, tannins, terpenoids, flavonoids, glycosides, phenols, alkaloids, sterols, and coumarins.

Test for saponins: Aqueous and 80% methanol extracts (0.5 g each) were dissolved in 5 ml of distilled water. Then, the mixture was vigorously shaken, and formation of stable foam confirmed the presence of saponins (Santhi and Sengottuvel, 2016).

Test for Tannins: About 50 mg of each extract was mixed with 10 ml of distilled water in a beaker. The mixture was then boiled and filtered. To the filtrate, 2- 3 drops of 0.1 percent $FeCl_3$ were added, and formation of brownish green color indicates tannins presence (Yamuna *et al*., 2017).

Test for terpenoids: A 2 ml of chloroform was added in 5 ml of AQ and ME extracts separately. To this solution, 3 ml of concentrated H_2SO_4 was cautiously added. A reddish-brown color at the interface shows the existence of terpenoids (Sheel *et al*., 2014).

Test for flavonoids: In 3 ml of both extracts, 1 ml of 10% NaOH was added. An intense yellow color was appeared and turned colorless on the addition of dilute HCl, indicates the presence of flavonoids (Ali *et al*., 2018).

Test for cardiac glycosides: In 5 ml of AQ and ME extracts, 2 ml of glacial acetic acid, a drop of $FeCl_3$ and one milliliter of conc. H_2SO_4 was carefully added. The presence of a brown ring at the interface confirms glycosides existence in the extracts (Yamuna *et al*., 2017).

Test for Steroids: About 2 ml of AQ and ME extracts was dissolved in 2 ml of chloroform, and 2 ml of conc. H_2SO_4 was added. Then, red color formation in the lower chloroform layer and greenish yellow fluorescent in the acid layer indicates the presence of steroids (Sheel *et al*., 2014).

Test for phenols: About 0.5 g of both extracts were dissolved in 5ml of distilled water. Then few drops of 5% $FeCl_3$ were added and appearance of dark green color confirms the existence of phenols (Philosia and Dhivya, 2017).

Test for alkaloids: AQ and ME extracts of 3 ml of each was warmed in 2% HCl for 20 minutes and filtered. Few drops of reagents were added separately. The formation of a creamy- white precipitate with Mayer's reagent or a reddish-brown precipitate with Wagner's reagent confirms the presence of alkaloids (Sheel *et al*., 2014; Santhi and Sengottuvel, 2016).

Test for anthraquinones: Each extract of 0.5 g weight was mixed with chloroform (5 ml), shaken for 5 minutes, and filtered. Then, it was shaken with an equal amount of 10% of ammonia solution. A red or pink violet color in the ammonia layer indicates the existence of anthraquinones (Mbahi *et al*., 2018).

Test for coumarins: In 1 ml of each extract, 1 ml of 10% NaOH was added and yellow color formation confirms the presence of coumarins (Ali *et al*., 2018).

3.10. Quantification of phytochemical constituents

Total flavonoids, phenols and alkaloids content were quantified as follows.

3.10.1. Total phenols content

Total phenol contents (TPC) of aqueous and 80% methanol extracts were estimated by Folin-Ciocalteu method (María *et al*., 2018). A linear calibration curve (Figure 3) was plotted using gallic acid at various concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/ml. About 1 ml of each extract (100 µg/ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent (1:10) and incubated for 8 minutes. Then, 2 ml of sodium carbonate (7.5%) was added, and the solutions were mixed well and incubated for 30 min at ambient temperature. The absorbance was then recorded with a UV-visible spectrophotometer (Unico UV-2100, USA) at 765 nm. The standard (gallic acid) and blank solutions were prepared using the same procedure. TPC was calculated for each extract using the following formula (Siddiqui *et al*., 2017) and reported as mg of gallic acid equivalent per gram of extract. The procedure was performed in triplicate and the average result was taken.

$$\text{TPC} = \frac{CV}{M}$$

Where TPC = total phenolic content in mg/g, C = concentration (mg/ml) of gallic acid obtained from calibration curve, V = volume (ml) of extract, M = weight of extract in gram.

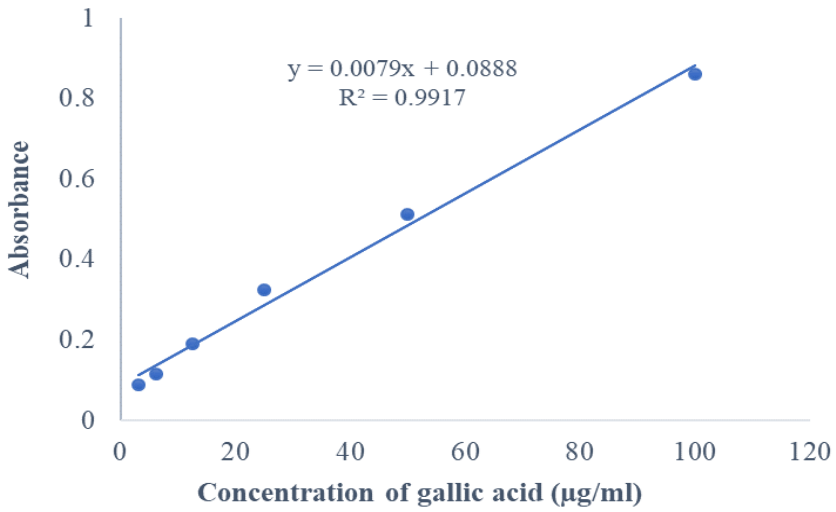

Figure 3: Calibration curve for gallic acid standard solution

3.10.2. Total flavonoids content

Estimation of total flavonoids content (TFC) of both AQ and ME extracts was carried out by using aluminum chloride colorimetric method (Durai *et al*., 2016). Quercetin was used as standard and serial concentrations of 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml were prepared in methanol to establish the calibration curve (Figure 4). In each test tube containing the standard (quercetin), 0.3 ml of $NaNO_2$ (5%) was added and incubated for five minutes. Next, 0.3ml of 10% $AlCl_3$ was added and incubated again for 5 minutes. Following that, 2 ml of 1M NaOH was added, and the mixture was diluted to 10 ml with distilled water. Finally, the mixture was left for 30 minutes at an ambient temperature. The same procedure was repeated for both extracts (1mg/ml) and blank solutions. The absorbance of standard, extracts (AQ and ME), and blank solutions was measured using a UV-visible spectrophotometer (Unico UV-2100, USA) at 510 nm. The assay was conducted in triplicate, and the average result was recorded. TFC was expressed in terms of mg of quercetin equivalent per gram of extracts.

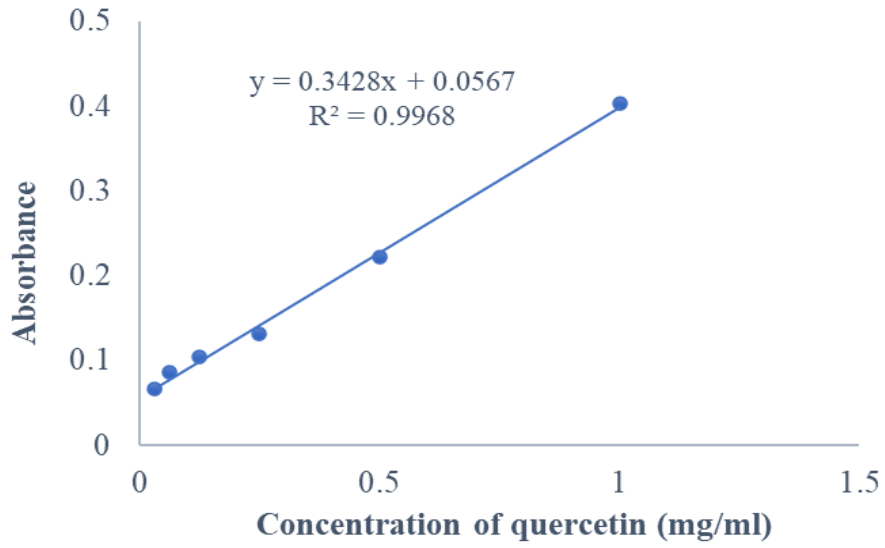

Figure 4: Calibration curve for quercetin standard solution

3.10.3. Total alkaloids content

Alkaloid content of both AQ and ME extracts was estimated using atropine as a standard (Ajanal *et al*., 2012). Atropine was dissolved in methanol (10 mg/10ml) with successive dilutions of 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml. Two milliliters of each extract (1 mg/ml) were dissolved in 2N HCl and filtered. One milliliter of this filtered extract was transferred to separating funnel, washed twice with chloroform (10 ml), and the chloroform extract was discarded. The pH of the remaining solution was neutralized with 0.1 M NaOH. To this, 5 ml of bromocresol green (BCG) solution and 5 ml of phosphate buffer were added. This solution was vigorously shaken, extracted twice with 8 ml of chloroform, and collected in 10 ml test tube. Chloroform was added until the volume reach to the mark. As described for extracts, a set of standard atropine solutions and a blank solution (methanol) were prepared. The absorbance of extracts, atropine and blank solutions was measured at 470 nm using a UV-visible spectrophotometer (Unico UV-2100, USA). Total alkaloids content was quantified as mg of atropine equivalent per gram (AE/g) of extracts using a linear calibration curve (Figure 5). All procedures were carried out in triplicate.

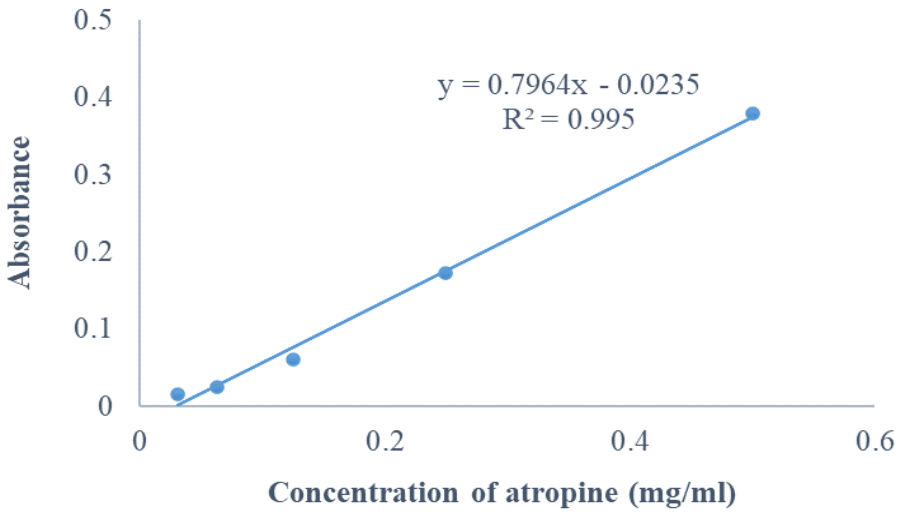

Figure 5: Calibration curve for atropine standard solution

3.11. Statistical analysis

The results of the experiment were analyzed with a Statistical Package for the Social Sciences (SPSS) window version 26 statistical software and expressed as mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) followed by a post hoc Tukey test was employed to compare differences between groups. At 95% confidence interval, p-value less than 0.05 ($p<0.05$) was considered as statistically significant. Linear regression was used to determine correlation coefficient (R^2) where applicable. The data were presented in tables and figures.

4. Results

4.1. Acute toxicity study

The acute oral toxicity study for both aqueous and 80% methanol extracts of the leaves of *S. abyssinica* did not show any sign of toxicity or mortality at a limit test dose of 2000 mg/kg during the first 24 h as well as the subsequent 14 days of follow up period.

4.2. Effects of both extracts of *S. abyssinica* leaf in pylorus ligation-induced gastric ulcer

Pylorus ligation resulted visible ulcers in the negative control with an ulcer index of 11.2 ± 0.07. Pretreatment of rats with both AQ (R^2 = 0.969; $p<0.05$) and ME (R^2 = 0.959; $p<0.05$) extracts of *S. abyssinica* resulted in a dose-dependent ulcer index reduction. All doses of AQ and ME extracts showed a significant ($p<0.001$) reduction in ulcer index as compared to the negative control. Ulcer index for aqueous extract were found to be 9.03 ± 0.21, 5.55 ± 0.21, and 3.45 ± 0.08 at doses of 100, 200, and 400 mg/kg, respectively. Similarly, the ulcer index for methanol extract were found to be 8.87 ± 0.12, 5.23 ± 0.08, and 3.42 ± 0.05 at the dose indicated for aqueous extract, respectively. Moreover, 200 and 400 mg/kg of both extracts and OMP20 exhibited a significant ($p<0.001$) ulcer index reduction in comparison to 100 mg/kg of extracts (Table 1). Regarding mucin content, the doses of 200 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.001$) showed a significant increment with percentage increments of 52.42% and 59.2% in AQ, and 53.71% and 60.66% in ME extracts, respectively, as compared to the negative control. There was a dose dependent stomach mucin content increment in AQ (R^2 = 0.955; $p<0.05$) and ME (R^2 = 0.973; $p<0.05$) extracts. The standard drug OMP20 significantly ($p<0.001$) increased the gastric wall mucus content, which is statistically comparable with higher doses of the extracts (Table 1).

As presented in Table 2, both extracts of *S. abyssinica* at all doses significantly reduced the gastric juice volume as compared to the negative control, and the reduction was highly significant ($p<0.001$) in higher dose of ME extract (ME400), which is comparable to OMP20 ($p<0.001$). Both AQ and ME extracts at 200 ($p<0.05$), and 400 mg/kg ($p<0.001$), as well as OMP20 ($p<0.001$) were able to significantly rise the gastric juice pH when compared to the negative control. Furthermore, AQ400 ($p<0.01$) and ME400 ($p<0.001$) produced a significant rise in gastric juice pH compared to AQ100. In comparison to ME100, higher doses of both extracts AQ400 ($p < 0.05$) and ME400 ($p < 0.01$) caused a considerable rise in gastric juice pH, and similarly, the standard drug ($p <$

0.001) caused higher gastric juice pH than both AQ100 and ME100 (Table 2). Table 2 also depicts that administration of both extracts at doses of 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$) resulted a significant decrease in free and total acidity when compared to the negative control, and the effect of higher doses was comparable with standard drug ($p<0.01$).

Table 1: Effects of *S. abyssinica* leaf extracts on ulcer index and mucin content against pylorus ligation-induced gastric ulcer.

Groups	Ulcer index	%ulcer inhibition	Mucin content (µg/g)	% increment of mucin
NC	11.2 ± 0.07	-	17.49± 2.39	-
OMP20	3.53 ± 0.13^{a3b3c3}	68.48	42.86 ± 5.32^{a3}	59.19
AQ100	9.03 ± 0.21^{a3}	19.8	30.3 ± 1.67	42.28
AQ200	5.55 ± 0.21^{a3b3c3}	50.45	36.76 ± 3.1^{a2}	52.42
AQ400	3.45 ± 0.08^{a3b3c3}	69.2	42.87 ± 4.47^{a3}	59.2
ME100	8.87 ± 0.12^{a3}	20.8	29.13 ± 1.44	39.96
ME200	5.23 ± 0.08^{a3b3c3}	53.3	37.78 ± 3.01^{a2}	53.71
ME400	3.42 ± 0.05^{a3b3c3}	69.46	44.46 ± 4.42^{a3}	60.66

Values are expressed as mean ± standard error of the mean for each group (n= 6), a: compared to NC, b: compared to AQ100, c: compared to ME100, [2:] $p<0.01$, [3:] $p<0.001$. NC = Negative control, OMP20 = Omeprazole 20 mg/kg, AQ = Aqueous extract, ME = 80% methanol extract, numbers (100, 200, and 400) are doses of extracts in mg/kg.

Table 2: Effects of *S. abyssinica* leaf extracts on gastric volume, pH, free and total acidity against pylorus ligation-induced gastric ulcer.

Groups	Gastric juice volume (ml)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)
NC	5.68 ± 0.17	1.78 ± 0.2	202.33 ± 61.39	411.67 ± 152.06
OMP20	1.6 ± 0.35^{a3}	4.74 ± 0.6^{a3b3c3}	46 ± 18.5^{a2}	87.5 ± 28.34^{a2}
AQ100	3.13 ± 0.66^{a1}	2.15 ± 0.06	97.5 ± 24.14	164.17 ± 37.43
AQ200	2.75 ± 0.82^{a2}	3.09 ± 0.23^{a1}	70.83 ± 5.54^{a1}	131.83 ± 15.55^{a1}
AQ400	2.47 ± 0.52^{a2}	4.1 ± 0.26^{a3b2c1}	46.83 ± 6.76^{a2}	92.17 ± 11.79^{a2}
ME100	3.13 ± 0.53^{a1}	2.67 ± 0.15	95 ± 10.88	165.83 ± 31.79
ME200	2.73 ± 0.43^{a2}	3.11 ± 0.26^{a1}	70 ± 8.56^{a1}	138.33 ± 17.78^{a1}

ME400	1.93 ± 0.53^{a3}	4.25±0.19^{a3b3c2}	46.17 ± 3.17^{a2}	93 ± 4.6^{a2}

Data are presented as mean ± standard error of the mean for each group (n= 6), a: compared to NC, b: compared to AQ100, c: compared to ME100, $^{1:}$ p<0.05, $^{2:}$ p<0.01, $^{3:}$ p<0.001. NC = Negative control, OMP20 = Omeprazole 20 mg/kg, AQ = Aqueous extract, ME = 80% methanol extract, mEq/L = Milliequivalent per liter, ml = Milliliter, numbers (100, 200, and 400) are doses of extracts in mg/kg.

As shown in Figure 6, pylorus ligation caused significant gastric lesions in the negative control group, whereas pretreatment of rats with both extracts of *S. abyssinica* (100, 200, and 400 mg/kg) and OMP20 decreased ulcer formation. Higher doses of extracts and the standard drug have almost similar effects.

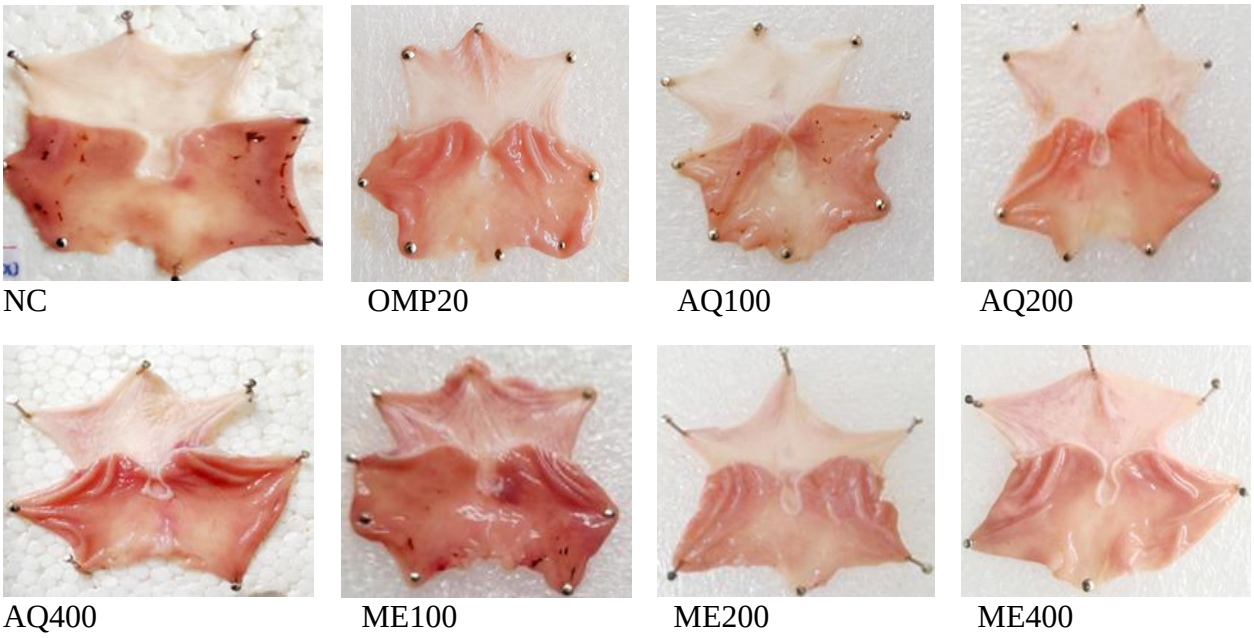

Figure 6: Gross effects of *S. abyssinica* leaf extracts against pylorus ligation-induced gastric ulcer. NC = Negative control, OMP = omeprazole, AQ = Aqueous extract, ME = 80% methanol extract, numbers (20, 100, 200, and 400) are doses in mg/kg.

4.3. Effects of both extracts of *S. abyssinica* leaf in indomethacin induced gastric ulcer

As presented in Table 3, indomethacin caused massive gastric ulcers with an ulcer index of 12.25 ± 0.23 in the control group. It was significantly reduced in rats pretreated with AQ and ME extract of *S. abyssinica* at doses of 100 (p<0.05), 200, and 400 (p<0.001) mg/kg, and the reduction was dose dependent with R^2 of 0.928; p<0.05 in AQ extract and R^2 of 0.977; p<0.01 in ME extract. The higher doses of both extracts have comparable ulcer index reduction with standard drug, M100 µg/kg. Concerning gastric mucin content, AQ200, and ME200 (p<0.01), AQ400, ME400, and

M100 ($p<0.001$) showed significant increment as compared to that of negative control. The % increment of mucin content for AQ extract was 38.3% and 45.52% at the doses of 200 and 400 mg/kg, respectively, but for ME extract it was 42.24% and 49.51% at the similar doses. ME400 ($p<0.05$), and M100 ($p<0.01$) produced a noticeable difference in mucin content when compared to AQ100 and ME100 (Table 3). The mucin content increment by extracts was dose dependent in AQ ($R^2 = 0.992$; $p<0.01$) and ME ($R^2 = 0.988$; $p<0.05$).

Table 3: Effects of *S. abyssinica* leaf extracts on ulcer index and mucin content against indomethacin induced gastric ulcer.

Groups	**Ulcer index**	**%ulcer inhibition**	**Mucin content (µg/g)**	**% increment of mucin**
NC	12.25 ± 0.23	-	32.8 ± 2.77	-
M100	3.57 ± 0.16^{a3b3c3}	70.86	65.73 ± 5.79^{a3b2c1}	50.1
AQ100	11.23 ± 0.2^{a1}	8.33	45.09 ± 3	27.26
AQ200	7.62 ± 0.31^{a3b3c3}	37.8	53.16 ± 3.71^{a2}	38.3
AQ400	3.62 ± 0.19^{a3b3c3}	70.45	60.21 ± 2.96^{a3}	45.52
ME100	11.17 ± 0.15^{a1}	8.82	47.92 ± 4.8	31.55
ME200	7.13 ± 0.21^{a3b3c3}	41.8	56.79 ± 2.31^{a2}	42.24
ME400	3.52 ± 0.13^{a3b3c3}	71.26	64.96 ± 3.42^{a3b1c1}	49.51

Values are presented as mean ± standard error of the mean for each group (n= 6), a: compared to NC, b: compared to AQ100, c: compared to ME100, [1:] $p<0.05$, [2:] $p<0.01$, [3:] $p<0.001$. NC = Negative control, M100 = Misoprostol 100 µg/kg, AQ = Aqueous extract, ME = 80% methanol extract, numbers (100, 200, and 400) are doses of extracts in mg/kg.

Figure 7 shows that the negative control received vehicle had a high number of ulcers. But animals pretreated with the extracts and a M100 had relatively fewer to no ulcers. Significant ulcer formation was not observed as the dose was increased from low to high.

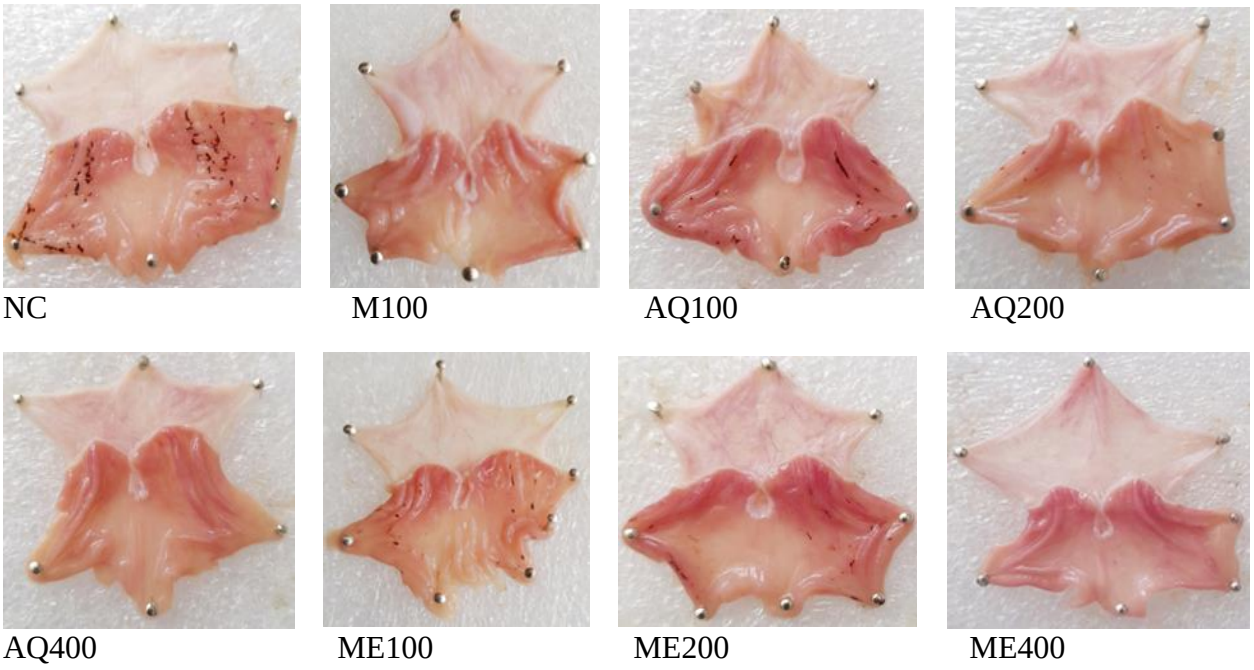

Figure 7: Gross effects of *S. abyssinica* leaf extracts against indomethacin-induced gastric ulcer. NC = Negative control, M100 = Misoprostol 100 µg/kg, AQ = Aqueous extract, ME = 80% methanol extract, numbers (100, 200, and 400) are doses of extracts in mg/kg.

4.4. Effects of both extracts of *S. abyssinica* leaf in ethanol-induced gastric ulcer

Table 4 summarizes the data obtained from ethanol-induced gastric ulcer model. Accordingly, oral administration of absolute ethanol (99.9%) exhibited deep or superficial erosions and hemorrhagic streaks as observed in vehicle treated group with an ulcer index of 12.9 ± 0.17, whereas pretreatment with extracts produced a statistically significant ($p< 0.001$) reduction at doses of 200 and 400 mg/kg, with ulcer indexes of 9.33 ± 0.26 and 5.38 ± 0.2 in AQ extract, and ulcer indexes of 9.13 ± 0.19 and 5.31 ± 0.17 in ME extract. An ulcer index reduction was dose dependent in AQ (R^2 of 0.976, $p< 0.05$) and ME (R^2 of 0.983, $p< 0.01$) extracts. The ulcer index for the standard drug, M100, was 3.65 ± 0.2. As compared to low dose (100 mg/kg) of both extracts, 200 and 400 mg/kg of both extracts and OMP20 showed a significant ($p<0.001$) reduction in ulcer index (Table 4). Furthermore, ethanol administration significantly decreased gastric mucin content in vehicle received rats. However, pretreatment with both extracts at doses of 200 ($p<0.05$) and 400 ($p<0.001$), as well as standard drug M100 ($p<0.001$) significantly enhanced the gastric wall mucin content compared to the negative control. Those higher dose of extracts also showed a significant ($p<0.01$) enhance in gastric mucin content when compared to AQ100 and ME100. M100 also increased mucin content much more than AQ100 ($p<0.001$) and ME100 ($p<0.01$). Both extracts

showed dose dependent gastric wall mucus content increment in AQ and ME with R^2 of 0.911, $p< 0.05$ and R^2 of 0.952, $p< 0.05$, respectively (Table 4).

Table 4: Effects of *S. abyssinica* leaf extracts on ulcer index and mucin content against ethanol-induced gastric ulcer.

Groups	Ulcer index	%ulcer inhibition	Mucin content (µg/g)	% increment of mucin
NC	12.9 ± 0.17	-	27.66 ± 4.82	-
M100	3.65 ± 0.2^{a3b3c3}	71.7	57.83 ± 2.89^{a3b3c2}	52.17
AQ100	12.18 ± 0.31	5.58	30.23 ± 2.79	8.5
AQ200	9.33 ± 0.26^{a3b3c3}	27.67	46.84 ± 2.79^{a1}	40.95
AQ400	5.38 ± 0.2^{a3b3c3}	58.29	54.96 ± 4.34^{a3b2c2}	49.67
ME100	11.95 ± 0.12	7.36	32.35 ± 3.93	14.5
ME200	9.13 ± 0.19^{a3b3c3}	29.22	46.91 ± 5.23^{a1}	41.04
ME400	5.31 ± 0.17^{a3b3c3}	58.84	56.95 ± 4.28^{a3b2c2}	51.43

Data are expressed as mean ± standard error of the mean for each group (n= 6), a: compared to NC, b: compared to AQ100, c: compared to ME100, $^{1:}$ $p<0.05$, $^{2:}$ $p<0.01$, $^{3:}$ $p<0.001$. NC = Negative control, M100 = Misoprostol 100 µg/kg, AQ = Aqueous extract, ME = 80% methanol extract, numbers (100, 200, and 400) are doses of extracts in mg/kg.

Gross examination showed that oral administration of absolute ethanol (1ml/200 g) caused superficial, hemorrhagic streaks, and deep ulcers in the negative control. Few hemorrhagic streaks or spots were also found in lower dose of extracts, but the number of ulcers decreased as the dose increased from low to high (Figure 8).

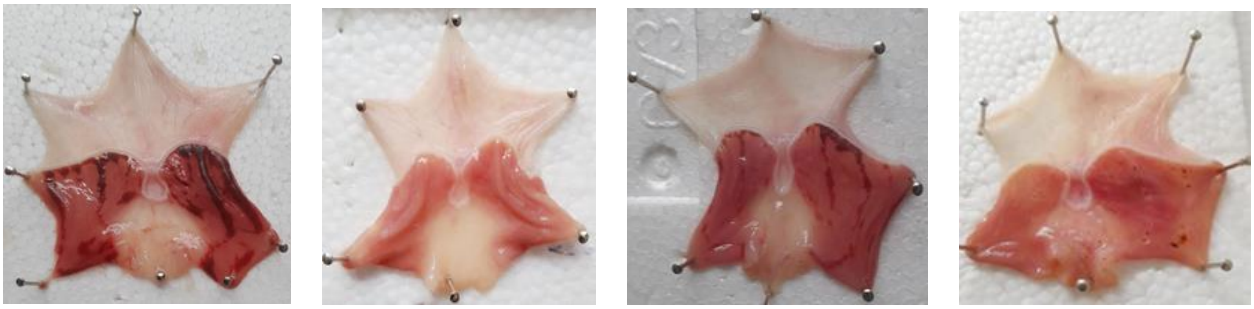

Figure 8: Gross effects of *S. abyssinica* leaf extracts against ethanol-induced gastric ulcer. NC = Negative control, M100 = Misoprostol 100 µg/kg, AQ = Aqueous extract, ME = 80% methanol extract, numbers (100, 200, and 400) are doses of extracts in mg/kg.

4.5. Phytochemical screening and quantification of phytochemical constituents

The phytochemical screening of both AQ and ME extracts of *S. abyssinica* leaf showed the presence of saponins, phenols, flavonoids, tannins, alkaloids, and coumarins as presented in Table 5. Moreover, the total phenols, flavonoids, and alkaloids content were quantified. Accordingly, the total phenols content was found to be 146.58 mg GAE/g and 189 mg GAE/g in AQ and ME extracts, respectively. Total flavonoids content was found to be 125.88 mg QE/g in AQ and 160.84 mg QE/g in ME extract. In addition, AQ and ME extracts contained 53.9 mg AE/g and 165.09 mg AE/g of total alkaloids, respectively (Table 5).

Table 5: Phytochemical screening of both extracts of *S. abyssinica* leaf and quantification of total phenols, flavonoids, and alkaloids.

Secondary metabolites	AQ	Quantity	ME	Quantity
Saponins	+	ND	+	ND
Phenols	+	146.58 mg GAE/g	+	189 mg GAE/g
Flavonoids	+	125.88 mg QE/g	+	160.84 mg QE/g
Alkaloids	+	53.9 mg AE/g	+	165.09 mg AE/g

Tannins	+	ND	+	ND
Steroids	-	ND	-	ND
Terpenoids	-	ND	-	ND
Anthroquinones	-	ND	-	ND
Cardiac glycosides	-	ND	-	ND
Coumarins	+	ND	+	ND

+ indicates the presence, - indicates the absence, ND: not determined, AQ: Aqueous extract, ME: 80% methanol extract, QE: Quercetin equivalent, GAE: Gallic acid equivalent, AE: Atropine equivalent, mg: milligram, g: gram.

5. Discussion

In Ethiopia, several medicinal plants have been identified and reported for their use in the treatment of PUD, but only a small number of plants have been scientifically studied for safety and efficacy in animal models (Tadesse *et al*., 2022). Therefore, the current study was conducted to evaluate the safety and efficacy of *S. abyssinica* leaf extracts claimed for the management of gastritis using animal models.

The acute toxicity study confirmed that both AQ and ME extracts of *S. abyssinica* leaf did not show any acute toxicity or mortality up to14 days at a dose of 2000 mg/kg. Thus, ingestion of *S. abyssinica* leaf for any of its medicinal effects would be supposed to be safe. Furthermore, the data suggested that this plant's leaf had a wider safety margin in which lethal dose is much more than 2000 mg/kg.

There are several models for evaluating antiulcer drugs (Adinortey *et al*., 2013). The pylorus ligation, indomethacin, and ethanol induced gastric ulcer models were selected to evaluate the gastroprotective effects of both AQ and ME extracts at three doses of 100, 200, and 400 mg/kg on parameters such as ulcer index, mucin content, gastric juice volume, pH, free, and total acidity.

Pylorus ligation is a commonly employed model for assessing the anti-secretory and cytoprotective effects of drugs that reduce gastric aggressive factor secretion and increase mucus production, respectively. Ulcers induced by pyloric ligation are due to accumulation of gastric secretion such as acid and pepsin, resulting to auto-digestion of stomach mucosa (Adinortey *et al*., 2013). In this model, both extracts showed a dose dependent reduction in ulcer index, and the effect of higher doses was more significant and comparable to a standard drug, omeprazole. In terms of mucin content, both extracts failed to show a significant increase in gastric mucin content at lower dose, but the medium and higher doses exhibited a substantial increase in gastric mucin content compared to the negative control. The effect is more significant with higher doses, probably due to increased concentration of active ingredients. It is interesting to note that the plant seems to have cytoprotective effects.

Accumulation of gastric acid/pepsin is an important aggressive agent for the formation of ulcers in the pylorus ligation model. This was observed in the vehicle administered group of animals, which had increased gastric juice volume and acidity as well as decreased gastric juice pH.

However, oral administration of both extracts at all doses exhibited a statistically significant reduction of gastric juice secretion as compared to the negative control. Concerning pH and acidity, a low dose of extracts failed to show a meaningful increase in gastric juice pH and a reduction in free and total acidity, while medium and higher doses showed a remarkable increment in pH and a decrease in acidity when compared to the vehicle received group. This demonstrates that the low dose of extracts might not be sufficient for gastric acid neutralization compared to the middle and higher doses, and the effect offered by the higher dose of extracts was comparable to antiulcer drug omeprazole, which is an irreversible proton pump inhibitor. This suggests that this plant may have potential anti-secretory activity.

As revealed in the phytochemical study, both extracts comprised different phytoconstituents including flavonoids, alkaloids, coumarins, and phenols which could justify the anti-secretory activity of *S. abyssinica*. Flavonoids have been well studied for their gastroprotective potential such as anti-secretory, cytoprotective, antioxidant, anti-inflammatory, and antibacterial effects (Perveen *et al*., 2018; Zhang *et al*., 2020). Their anti-secretory effect may be due to their antihistaminic properties, which reduce histamine levels while also limiting histamine release from gastric mast cells by inhibition of histidine decarboxylase and inhibiting gastric H+/K+ ATPase, resulting in decreased gastric acid secretion (Borrelli,and Izzo, 2000; de Lira Mota *et al*., 2009; Sharath *et al*., 2015). Likewise, alkaloids have the same effect via H_2-receptor antagonism and anti-cholinergic action (Mosaddik and Alam, 2000). Another mechanism by which alkaloids might have anti-secretory effect is mainly by blocking H+/K+-ATPase activity and gastrin secretion (Zhang *et al*., 2014). In addition, phenolic substances have the capability to inhibit the H+, K+-ATPase action (Siddaraju and Dharmesh, 2007). The other phytoconstituents, such as coumarins, may reduce gastric secretion through an anticholinergic mechanism or by interfering with intracellular processes that are related to acid secretion (Bighetti *et al*., 2005).

NSAIDs are known to induce gastric ulcers by inhibiting cyclooxygenase which is important in the biosynthesis of prostaglandins involved in maintaining the integrity of the gastric mucosa (Takeuchi, 2012), and indomethacin is a commonly used NSAID to induce gastric ulcer in the experimental animal (Suleyman *et al*., 2010). In this study, 40 mg/kg of indomethacin was administered for gastric ulcer induction. The model was employed to assess the cytoprotective effects of extracts.

Following ulcer induction with indomethacin, there were visible and noticeable gastric ulcers in the negative control as confirmed by a higher ulcer index and a low mucin content. However, extract pretreated groups showed a dose dependent decrease in ulcer index and an increase in mucin content compared to ulcerated group. The fact that indomethacin administration makes the stomach more susceptible to mucosal damage may be due to the suppression of endogenous PG synthesis (Inas *et al*., 2011). Nevertheless, exogenous PG, particularly the E series, protect gastrointestinal mucosa from injury induced by a broad range of irritants (Brzozowski *et al*., 2005). This was observed in the positive control group of rats treated with misoprostol. In accordance with this, the gastroprotective activity offered by extracts of AQ400 and ME400 was comparable to standard medication (M100) pretreated animals. Hence, cytoprotection could be a plausible mechanism for *S. abyssinica*'s gastric ulcer preventive activity.

The gastroprotective effect of the plant might be due to its effects in enhancing the mucus and HCO_3 secretion, stimulation of prostaglandin production, and gastric blood flow. These may be arbitrated through phenols, flavonoids, tannins, and saponins found in the extracts. Phenolic substances boost PGE synthesis by acting as co-substrates in the peroxidase reaction (Bansal and Goel, 2012). Similarly, flavonoids are highly gastroprotective, probably by increasing gastric PG content, gastric mucus secretion and gastric blood flow (de Lira Mota *et al*., 2009). Saponins may contribute to antiulcer activity by promoting PG and mucus formation in the stomach mucosa (Andargie *et al*., 2022; Yismaw *et al*., 2020). Moreover, tannins have astringent properties and react with the tissue proteins with which they come into contact. They produce a tannin-protein complex that protects the stomach mucosa by enhancing resistance to chemical and mechanical harm or irritation (De Jesus *et al*., 2012).

Ethanol-induced gastric ulcer model was also used to assess the gastroprotective activity of both extracts of *S. abyssinica*. It is a typical experimental model for the preclinical assessment of agents with potential anti-ulcer activity (Arab *et al*., 2015) which have also cytoprotective and/or antioxidant activities. Ethanol easily penetrates the stomach mucosa due to its capacity to dissolve the protective mucus and expose the mucosa to the hydrolytic and proteolytic effects of HCl and pepsin (Adinortey *et al*., 2013). Additionally, ethanol administration causes necrotic gastric injury and inflammatory cell infiltration, as well as a decrease in the secretion of gastric mucus, bicarbonate, and nitric oxide (Sistani Karampour *et al*., 2019; Zhou *et al*., 2020).

In this ethanol induced gastric ulcer model, ulcer index was found to be high in the control group. However, pretreatment with medium and higher dosages of extracts dramatically reduced ulcer index compared to the negative control. In addition, the determination of gastroprotective mucin content in this model revealed a significant decrease in the vehicle administered group. Meanwhile, a considerable increment of gastric mucin was observed in rats treated with misoprostol and extracts at the doses of 200 and 400 mg/kg. This indicates that the antiulcer effect of *S. abyssinica* is mediated partly by preserving gastric mucus.

Furthermore, the cytoprotective effect of this plant could be related to its antioxidant activities. Indeed, exposure of gastric mucosa to ethanol induces oxidative stress by increasing malondialdehyde (MDA) production and reducing antioxidant enzymes such as SOD, CAT, and GSH formation (Al Batran *et al*., 2013; Zhou *et al*., 2020). Conversely, a report from Zhou *et al*. (2020) showed that pretreatment of rats with gallic acid, a phenolic compound, revealed a considerable rise of SOD, GSH, and CAT levels, which suggests its antioxidant potential against ethanol induced gastric ulcer. The antioxidant properties of phenols are mainly related to their ability to scavenge free radicals due to the presence of a hydroxyl functional group. Thus, phenolic content of plants might contribute its pharmacological activity due to its antioxidant action (Tosun *et al*., 2009). Another bioactive component, flavonoids, also exert an antioxidant effect by scavenging free radicals, protecting and activating antioxidant enzymes, inhibiting oxidizing enzymes, and preventing lipid peroxidation (Cheng *et al*., 2013; Zhang *et al*., 2020). In line with these, *S. abyssinica* may have antioxidant activities due to the presence of high content of flavonoids and phenols in both AQ and ME extracts. Therefore, the antioxidant properties of plant phytoconstituents might be beneficial in gastric mucosa protection.

Gastric mucus serves a crucial role in the defensive mechanism against stomach ulcers. It forms adherent layer that covers the gastric mucosa, preserves a near neutral pH at the mucosal surface, and provides a physical barrier against luminal pepsin (Allen and Flemström, 2005). The weakening of mucus barrier is directly responsible for gastric mucosa damage (Sidahmed *et al*., 2019). The quality and quantity of gastric mucus secretion are the crucial indicators of mucosal defense barrier status against undesirable effects of acid and pepsin (Zakaria *et al*., 2014). In the present study, as mentioned above, gastric mucin content was determined in the three models. The pylorus ligation, indomethacin, and ethanol significantly decreased gastric mucus/mucin

production, but administration of each extract significantly increased the amount of mucus secretion, specifically, at the middle and higher doses, indicating increased mucin production might be one of important factor in the gastroprotective activity of *S. abyssinica.*

The therapeutic effect of medicinal plants is mainly associated with the presence of phytochemical constituents, such as flavonoids, tannins, phenols, terpenoids, saponins, and alkaloids (Ahmad *et al*., 2014; Sharifi-Rad *et al*., 2018). As a result, the phytochemical screening test used to identify those constituents revealed the existence of saponins, flavonoids, phenols, alkaloids, tannins, and coumarins in both AQ and ME extracts. In addition, quantitative analysis of both extracts showed the presence of high contents of total phenols, flavonoids, and alkaloids. ME extract, on the other hand, had more phenols, flavonoids, and alkaloids than AQ extract. This may explain, at least in part, the slight difference in activity between the two extracts.

6. Conclusion

The aqueous and 80% methanol leaf extracts of *S. abyssinica* possessed remarkable gastroprotective activities in pylorus ligation, indomethacin, and ethanol-induced gastric ulcer models. The gastroprotective effects of *S. abyssinica* leaf extracts could be related to the existence of high content of phytoconstituents like phenols, flavonoids, and alkaloids. The precise mode of action of extracts is unknown, but anti-secretory and cytoprotective effects may be the possible mechanisms of gastroprotective activity of the plant. The findings of this study provide a scientific support for the traditional use of *S. abyssinica* leaf to treat gastritis.

7. Recommendation

Based on the findings of this study, the following are suggested for further study:

- Sub-acute and chronic toxicity study of the plant extract should be carried out;
- Fractionation of the crude extract and their gastroprotective activity study should be carried out;
- Further study should be done in other models including anti-*H. pylori;*
- Further study should be done to isolate and identify pharmacologically active components responsible for the gastroprotective activity of the extracts;
- Further studies should be carried out to confirm the precise mechanism of action of the gastroprotective activity of the extracts.

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