GASTROPROTECTIVE EFFECT OF METHANOL LEAF EXTRACT OF *MALLOTUS ROXBURGHIANUS* MÜLL.ARG. AND FRUIT EXTRACT OF *GARCINIA LANCEIFOLIA* ROXB. IN GASTRIC ULCERS IN WISTAR ALBINO RATS.

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Gastroprotective effect of methanol leaf extract of *Mallotus roxburghianus* Müll.Arg. and fruit extract of *Garcinia lanceifolia* Roxb. in gastric ulcers in Wistar albino rats.

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IN PARTIAL FULFILLMENT OF THE REQUIRMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY OF MIZORAM UNIVERSITY.

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I certify that the thesis entitled **"Gastroprotective effect of methanol leaf extract of *Mallotus roxburghianus* Mull.Arg. and fruit extract of *Garcinia lanceifolia* Roxb. in gastric ulcers in Wistar albino rats"** submitted to Mizoram University for the award of the degree of **Doctor of Philosophy** in **Zoology** by **Sagun Kumari** bearing **MZU Regn. No. 1600820** and **Ph.D. Regn No. MZU/Ph.D/1009 of 26.05.2017** is a record of dissertation work carried out under my guidance and supervision, and that this work has not formed the basis for the award of any degree, diploma or other titles in this university or any other university or institution of higher learning.

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MAY, 2024

I **Sagun Kumari**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

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CHAPTER 1

INTRODUCTION

1.1 GASTRIC ULCER AND ITS MAGNITUDE

Gastric ulcer (GU) disease is a well-known global health problem characterized by the presence of benign lesions/open sores of the gastric mucosa of stomach that might reach the muscularis mucosa or beyond (Escobedo-Hinojosa et al., 2018). Gastric ulcer is a one of the most prevalent worldwide gastrointestinal ailments and its prevalence is dominant in the Asian countries (Dutta et al., 2012). According to reports, GU has a 5–10% lifetime frequency in the general population and an annual incidence of 0.1-0.3 % (Lanas and Chan, 2017). Thus GU is a crucial health issue affects an estimated 10% of the world's population and has a significant negative impact on millions of people's quality of life (Havens et al., 2018). Progression of GU results in hemorrhage (20%) and perforation (7%), affecting 87.4 million of the world population and resulted in 0.267 million deaths in 2015 (Wang et al., 2016). Although, the prevalence of gastric ulcer varies greatly based on age, gender, and geography, it is nevertheless a highly prevalent ailment and a significant public health issue worldwide (Xie et al., 2022). The prevalence of duodenal ulcer is dominant in Western population while gastric ulcer is more common in the Asian countries, gastric and duodenal ulcer together referred as peptic ulcer (Yuan et al., 2006). Peptic ulcer is most prevalent gastrointestinal ailment affects 40% of developed countries and 80% of developing countries (Ajeigbe et al., 2014). The greatest risk of stomach cancer comes from long-term occurrence with GU or duodenal ulcer (Hansson et al., 1996). According to reports, type 2 diabetes, mental health conditions, and liver cirrhosis are all more likely in people with GU (Rahman et al., 2020). The peptic ulcer condition is extremely common among people worldwide, to the point where some academics have dubbed it the new emerging pandemic of the twenty-first century (Sumbul et al., 2011).

1.2 PATHOGENESIS OF GASTRIC ULCER

The pathogenesis of gastric ulcers is complex with lesions in mucus layer may be associated one or variety of internal and external factors. The GU results from an imbalance between various damaging/injurious and protective components (Gugliandolo et al., 2021). Aggressive/injurious factors includes endogenous pathogenic agents and events like elevated gastric hydrochloric acid, pepsin secretion, lipid peroxidation, inherited predisposition and excessive reactive oxygen species (ROS) production (Thorsen et al., 2013; Graham 2014,) and exogenous factors such as alcohol consumption, physical and psychological stress, tobacco and cigarette smoking, long-term use of non–steroid anti-inflammatory drugs(NSAIDs), antiplatelet agents (acetyl salicylic acid), immunosuppressive medications, serotonin reuptake inhibitors, pollutants, pesticides, heavy metals and gram-negative microaerophilic bacterial infection e.g. *Helicobacter pylori* (Ji et al., 2012; Mousavi et al., 2020; De Araújo et al., 2021). While protective factors include, mucosal barrier such as gastric wall mucus (GWM), mucus (mucin secretion) and bicarbonate production, biosynthesis of gastroprotective prostaglandin, nitric oxide (NO), growth factors, adequate blood flow, normal tissue microcirculation, surface phospholipids, and endogenous antioxidants (Al Batran et al., 2013; Farzaei et al., 2015). Mucin secretion and interaction of acid and pepsin are the main components to protect the gastric mucosa (GM) from lesions and used as indicators to assess the GU complications (Al Batranet al., 2013).

When left untreated, gastric ulcers can penetrate the stomach's muscle layers and produce acute lesions in the gastric mucosa. Thus, they can cause chronic inflammation and may result in problems like hemorrhages, perforations and obstructions (Proctor and Deans, 2014). The significant morbidity and mortality of GU disease are attributed to high healthcare expenses and above mentioned life-threatening complications (Escobedo-Hinojosa et al., 2018). In addition, the illness severely affects patients' daily lives and causes them tremendous physical and emotional agony. The majority of peptic ulcer sufferers experience nausea, pain, or abdominal distress. The most typical sign of gastric and duodenal ulcers is abdominal

pain. After food eating, this pain is characterized by a burning sensation (Sharifi-Rad et al., 2018).

An increase in stomach acid levels in the gastro intestinal tract (GIT) is linked to gastric hyperacidity, which corrodes the linings of stomach. This overabundance of acid production can cause acute or chronic gastritis, which is associated with inflammation, irritation, or erosion of the stomach mucosa later leads to gastric ulcer (Patel et al., 2018).

It is believed that multiple factors contribute to the etiology of gastric ulcers. *Helicobacter pylori* has been identified as one of important etiologic factors in gastric ulcer (Cohen, 2000), yet the bacterium by itself is not enough to cause major ulceration in the gastric tissue (Liu and Cho, 2000). Important exogenous factors such as NSAIDs and/or ethanol can cause acute erosive gastritis (Kholmurodovich, 2022; Li et al., 2016), may potentially cause more harm to the gastrointestinal mucosa (Liu and Cho, 2000).

Long-term NSAIDs-induced gastric damage is the major side effect and pathophysiology of gastric ulcer. The NSAID is predominantly ascribed to their ability to reduce prostaglandin (PG) production through the inhibition of cyclooxygenases (COX). The cyclo-oxygenase enzymes isoforms COX-1 and COX-2 are both crucial for mucosal defense. Prostacyclin (PGI2) and PGE2 are prostaglandins (PGs) that have protective benefits for the stomach mucosa, and COX-1 is essential for their production. PGs play important roles in modulating the mucosal integrity and various functions of the gastrointestinal tract, such as stimulating the secretion of bicarbonate and mucus, maintaining the blood flow of the mucosa, and regulating mucosal cell renewal (Takeuchi and Amagase, 2018). Additionally, tissue oxidation, lipid peroxidation, and apoptosis or programmed cell death are believed to be other main factors that contribute to the pathogenesis of NSAID-induced gastric damage (Chen et al., 2016).

The stomach mucosa is also known to be damaged by ethanol and excessive ethanol consumption is the primary cause of GUs in humans can cause acute gastric mucosal injury (Li et al., 2016). As a significant contributing element, ethanol

damages the stomach mucosa by a variety of intricate processes, ranging from direct contact to indirect biological harm brought on by its metabolite acetaldehyde. In the meantime, the buildup of acetaldehyde digested from 10% of alcohol in the stomach causes indirect harm to the gastric mucosal cells by inducing oxidative stress and inflammatory reactions since ALDH (acetaldehyde dehydrogenase) is absent (Simon et al., 2022). Dehydration, cytotoxicity, and disruption of mucosal cellular membranes are among the immediate impacts, which lead to the inflammatory cascade spreading (Akanda et al., 2017). This may result in both the mitochondria-dependent and mitochondria-independent pathways of cellular death. Ethanol-induced necrotic lesions in the GWM produce free radical species, which can cause GWM obstruction, decrease the bicarbonate secretion and mucus production and in turn damage the gastric system (Al Batran et al., 2013). The synthesis of pro-oxidative, pro-inflammatory, and free radical enzymes and molecules as well as free radicals, due to excessive ethanol exposure causes activation of neutrophil infiltration into the area of injury, which damages the stomach mucosa and causes oxidative stress (Hussein et al., 2016). At cellular level ethanol induces imbalance between proliferation and apoptosis in the gastric tissue could be the decisive factors in the level of gastric mucosa damage (Liu and Cho, 2000). It was shown that persistent alcohol consumption causes the pro-inflammatory cytokines such as tumor necrosis factor- (TNF), interleukin-1β (IL1β), and IL-6 to have a role in the production of buccal mucosal apoptosis (Amirshahrokhi and Khalili, 2015). Additionally, ethanol efficiently lowers the level of NO required for physiological activity in the stomach mucosa and slows the flow of gastric blood, leading to hemorrhagic lesions and the resultant solubilization of gastric mucus components (Konturek et al., 2017). The most frequent cause of stomach mucosa injury, according to recent research studies, is excessive, uncontrolled alcohol use (Zheng et al., 2017). Compared to nondrinking populations, the incidence of stomach mucosal lesions, such as gastritis, gastric ulcers, and even gastric cancer, is much higher in the drinking population (Strate et al., 2016).

1.3 ETHANOL INDUCED RAT MODEL FOR ANTI-ULCER

Long term high ethanol consumption is being cited one most prominent reason for gastric ulcer and individuals consuming alcohol at high risk for ulcer (Zheng et al., 2017). Ethanol has the ability to digest gastric mucosal layer and further exposes it to the hydrolytic and proteolytic actions of gastric secreted hydrochloric acid and pepsin (Rahman et al., 2020). Thus ethanol causes microvascular endothelium damage by decreasing blood flow and raising proinflammatory cytokine and reactive oxygen species (ROS) generation, it lowers antioxidant levels inside cells (Padovan et al., 2023). Rat is being used as a most common experimental model for testing anti-ulcer drugs/medicinal plant extracts due followings reasons. In the testing of anti-ulcer drugs large numbers of animals are required to produce reproducible data on the efficacy of target drugs/plant extracts. Rat is a good candidate as it has good reproductive potential and less generation. Can be managed, reproduce under animal conditions. Moreover, the stomach of the rat is anatomically and functionally similar and analogous to the human stomach (Jiminez et al., 2015). Rat stomach is differentiated into two parts namely upper non-secretory and lower glandular like human stomach. As a result, the procedure for testing anti-ulcer activity of various drugs, new compounds and plant extracts that is most frequently utilized is the ethanol-induced gastric ulcer in experimental rat animal model (Beiranvand, 2022).

1.4 CURRENT ALLOPATHIC MEDICATIONS FOR GASTRIC ULCER AND THEIR LIMITATIONS

A number of medications are used to treat stomach ulcers. The first optional medication used to treat dyspepsia and heartburn is antacids. This group is ineffective in stopping or treating stomach ulcers, but it can be helpful in two ways: either by protecting the stomach's mucosal layer by increasing bicarbonate and mucus secretions or by neutralizing acidity inside the stomach lumen by combining three basic compounds: calcium, magnesium, and aluminum (Shorrock and Rees,1991; Gobinath, 2014).

Antibiotics can be used to treat stomach ulcers brought on by an *H. pylori* bacterial infection. Amoxicillin is a popular positive reference medication for assessing antibacterial activity against many bacteria, including *H. pylori*. It is an efficient antibiotic that reduces bacterial growth (Fu et al., 2021). Prior to the proton pump inhibitors (PPI) and antibiotics that target *H. pylori*, a class of medications known as H_2 blockers, which includes cimetidine and ranitidine, was the primary treatment for stomach ulcers (Feely and Wormsley, 1983). H_2 blocker inhibits histamine, a cytokine molecule produced by the body that encourages acid release in the stomach; they function as suppressors of acid production (Untersmayr, 2015).

PPIs, or proton pump inhibitors, are the most often prescribed medication for stomach ulcers patient (Savarino et al., 2017). The mode of action involves inhibiting H+/K+ ATPase, the key enzyme regulates acid secretions from stomach glands' parietal cells, in order to prevent the release of stomach acid (Mullin et al, 2009; Zhang et al., 2020). The PPIs that are currently approved for the treatment and prevention of ulcers include rabeprazole, lansoprazole, esomeprazole, and omeprazole (Strand et al., 2017). Each of these medications has several side effects (Singh et al., 2018).

But using PPIs is not without its drawbacks. Primary adverse effects of PPIs include headache, constipation, diarrhea, nausea, and rash; these are usually on the order of 1% to 5 % (Chubineh and Birk, 2012). Clinical assessment of these medications revealed the possibility of drug interactions, unfavorable side effects, and relapses during ulcer treatment especially in elderly people(Scarpignato et al., 2016).These antiulcer modern medicines/agents do not permanently cure the ulcer but relieve symptoms via suppression of acid secretion and it long term uses may cause adverse side effects, ineffective and reduce the absorption of magnesium, vitamin B_{12}, calcium and iron which require an acidic gastric medium for bioavailability, gynecomastia, hypergastrinemia and hypercalcemia, diarrhea, kidney stones, osteoporosis, increased risk of enteric infections, altered metabolism of other medications, and formation of gastric polyps/carcinoid(Scarpignato et al., 2016; McQuaid, 2018; D'Silva et al., 2021). The use of PPIs results in decreased somatostatin release, decreased acid secretion, and consequently, increased gastrin

release from G-cells and hypergastrinemia. In as many as 7% to 10% of people receiving PPIs for a full year, stomach cells have the potential to become hyperplastic and develop fundic gland polyps (FGPs) (Chubineh and Birk, 2012). Long-term PPI use may predispose certain patients to the formation of neuroendocrine tumors, an issue brought up by hypergastrinemia (Chubineh and Birk, 2012).

1.5 MEDICINAL PLANTS AS AN ALTERNATIVE SOURCE OF PHYTOMEDICINE IN THE MANAGEMENT OF GASTRIC ULCER

The lack of safe and effective treatments for this widespread health issue drives the search for new classes of chemicals that have strong activity and a favorable safety profile. For millennia, nature has been regarded as the fundamental source for the development of novel, structurally varied medicinal medicines (Sen and Samanta, 2015). Stomach disease and disorder, including gastric ulcers; have been treated with medicinal plants and their secondary metabolites. Several secondary metabolites with distinct gastroprotective modes of action have been found in medicinal plants with gastroprotective properties (Singh et al., 2018). It has also been suggested that secondary metabolites have strong anti-inflammatory, anti-apoptotic, anti-lipid peroxidation, and antioxidant properties contribute to their gastroprotective effects (Singh et al., 2018; Raish et al., 2021; Shipa et al., 2022). Given that herbal remedies are thought to be less harmful, affordable, efficient, and relatively less toxic, they are safe to use in the treatment of ulcers. Plant based natural products can be used therapeutically, prophylactically, or in both capacities to demonstrate their antiulcerogenic properties. Several important medicinal plants are reported for their anti-ulcer activities such as *Aloe vera* (L.) Burm.f, *Anacardium humile* St. Hil., *Allophylus serratus* Kurz, *Butea frondosa* Roxb., *Carica papaya* Linn., *Coccinia grandis* Linn., *Cissus quadrangularis* L., *Curcuma longa* L., *Encholirium spectabile* Mart., *Gymnosporia rothiana*, *Lasianthera Africana* P. Beauv., *Mangifera indica* L., *Matricaria chamomilla* L., *Morus alba* Linn., *Mikania laevigata* Schultz Bip., *Ocimum sanctum* Linn., *Panax ginseng*, *Parkia platycephala* Benth., *Pausinystalia macroceras* (K. Schum.) Pierre ex Beille, *Piper betel* Linn., *Polyalthia longifolia* (Sonn.) Thwaites (PL), *Quassia amara* L., *Rhizophora mangle* L., *Sapindus*

trifoliatus L., *Solanum nigrum* L., *Syzygium aromaticum* L., *Terminalia chebula* Retz., *Zataria mul*tiflora, *Zingiber offic*inalis Roscoe, *Zizyphus oenoplia* (L.) Mill. (Borrelli and Izzo, 2000; Srinivas et al., 2013; Harsha et al., 2017; Sharifi-Rad; 2018; Gupta et al., 2021).

Medicinal plants contribute significantly as part of a long-standing traditional healthcare system intimately linked to economically marginalize rural people of Mizoram (Chinlampianga, 2011). There is great paucity of documentation and scientific validation of medicinal plants of Mizoram, NE-India. It is interesting to note that almost every plant is assigned the local name here, indicating their close relationship with the local people. So far, about 500 species under 383 genera have been recorded from the state, possessing medicinal and ethno-botanical uses (Singh et al., 2002; Sharma et al., 2001; Rai and Lalramnghinglova, 2010). Plant-based medicines represent a vast untapped resource that has shown enormous therapeutic potential. *Mallotus roxburghianus* Mull.Arg. (Euphorbiaceae) is native plants of Mizoram (India) found particularly in the tropical evergreen forests and mixed bamboo forests.

It is distributed in the region of Chittagong Hill tract of Bangladesh and Myanmar. The tribal people of Mizoram used the decoction of leaves for the treatment of ulcers, diabetes, fungal infections, and wound healing and so on. The ethanol extract of *M. roxburghianus* has been reported for its anti-diabetic and antioxidant activity (Lalhlenmawia et al., 2007; Roy et al., 2016). Methoxy benzoic acid, rhamnopyranoside, betulinic acid and bergenin will be isolated and identified from the leaves of *M. roxburghianus* (Rana et al., 2005). Berginin exhibits antihepatotoxic, antiulcerogenic, anti-HIV, antifungal, hepatoprotective, antiarrhythmic, neuroprotective, anti-inflammatory, immunomodulatory and burn wound healing properties (Patel et al., 2012). Free radical cytotoxicity of the gastrointestinal membrane is modulated by the antioxidant enzymes (Saeed AL-Wajeeh et al., 2016).

Garcinia lanceifolia is an important endemic medicinal plant of NE-India, frequently known as “Rupahi-thekera” (Assamese), “Pelte” (Mizo), “Rupohi tekera” (Mising),

belonging to the family Clusiaceae. The plant is well known for its ethnomedicinal values for stomachic & diuretic in Mizoram, pain reliever and hypoglycemic agents in Assam (Singh et al., 2002; Baruah 2007; Bora et al., 2014). The plant known harbor rich source of bioactive compounds such as phenolics, tannins, saponins, flavonoids & terpenoids, alkaloids with potential anti-oxidative, anti-bacterial, anti-inflammatory and antifungal properties (Chowdhury and Handique, 2012; Policegoudra et al., 2012; Bora et al., 2014).

Looking into the potential prospects of above mentioned ethno-medicinal plants, the present work was undertaken with following

1.6 OBJECTIVES:

- Detection and identification of phyto-compounds in *Mallotus roxburghianus* and *G. lanceifolia* by GC-MS/LC-MS.
- Evaluation of anti-ulcer and ulcer healing activities.
- Histopathological analysis and examine the extent of oxidative stress and the role of antioxidant enzymes in attenuation of gastric ulcer by *M. roxburghianus* and *G. lanceifolia*.
- Role of inflammatory and apoptotic genes and proteins in gastric ulcer formation by immunohistochemistry, immunoblotting and RT-PCR.

CHAPTER 2

REVIEW OF LITERATURE

2.1 MAJOR CAUSES OF GASTRIC ULCER

An erosion or lesion in the lining of the digestive tract is known as peptic ulcer disease (Urs et al., 2014). Depending on the part of gastrointestinal affected such as stomach and duodenum peptic ulcer further classified into gastric and duodenal ulcer respectively (Ravishankar et al., 2016). The structural integrity of stomach and duodenum is mainly affected and compromised in the ulcer by digestive action of stomach acid, pepsin and other etiological factors (Silva and de Sousa, 2011).

Gastric ulcer (GU) is characterized as a localized stomach breach accompanied by tissue degradation that may extend to the muscle layers i.e. muscularis mucosa (White and Carey, 2020). A stomach ulcer can cause a variety of symptoms, including bloating, vomiting, nausea, altered and loss of appetite, and burning or gnawing pain. The incidence of stomach ulcers varies significantly globally based on factors such as age, gender, and geography, yet they continue to be a foremost public health concern due to high healthcare expenditures (Groenen et al., 2009). Approximately 10% of people in the Western world suffer from stomach ulcer disease while 11.5% of people in Asia and the South Pacific have been diagnosed with stomach ulcers, making it one of the most common chronic diseases in the world's population (Barkun and Leontiadis, 2010).

In chronic case potentially fatal conditions such as bleeding, perforation, blockage, pyloric stenosis, and even may develop into malignant tumors which accounts for the significant morbidity and death rate linked to the illness (Jaiswal et al., 2021). The gastric ulcer pathophysiology is complex and multifactorial result due imbalance in the equilibrium between stomach’s mucosa aggressive and protective factors (Zatorski, 2017). The gastric mucosal integrity, mucus secretion, bicarbonate production, nitric oxide (NO), gastro protective prostaglandin synthesis, normal gastric motility, and adequate tissue microcirculation are among the gastrointestinal defense mechanisms (Serafim et al., 2020). While on the other hand, the noxious

factors include the secretion of gastric acid and pepsin, bile salts, reactive oxygen species (ROS), *Helicobacter pylori* infection, alcohol consumption, and prolonged use of non-steroidal anti-inflammatory (NSAID) medications (Silva and de Sousa, 2011; Ardalani et al., 2020).

Acid outputs are elevated in as many as one-third of patients with gastric ulcers. The increase in baseline acid output (BAO) may indicate that a sizable portion of patients suffer from stomach ulcers. Furthermore, some patients with gastric ulcers also show a rise in stomach acidity (Kotreka and Adeyeye, 2011).

NSAID use has been linked to the development of duodenal and stomach ulcers as well as gastrointestinal injuring (Ng and Chan, 2010). Within three months of beginning therapy, 4–10% of patients taking a daily therapeutic dose of NSAIDs develop stomach ulcers, with up to 1% of those ulcers being clinically severe (McCarthy, 1989). NSAID use is associated with an increased risk of stomach ulcers among older adults, women, and those who use the medicine frequently and for an extended period of time (Sostres et al., 2010).

Lifestyle factors, such as smoking, alcohol consumption can increase the chance of developing a gastric ulcer disease by speeding up the emptying of the stomach and reducing the production of pancreatic bicarbonate, which can lead to the ulcer (Mustafa et al., 2015). Drinking alcohol irritates the stomach mucosa, can lead to non-specific gastritis, and can also produce reflux, which is one of the main causes of stomach ulcers (Elseweidy, 2017).

A family history of stomach ulcers is present in over 20% of patients, compared to 5–10% in the control group. It's unclear why this genetic relationship exists. Gastric ulcers and familial hyperactive pepsinogenemia type I, a genetic trait that results in enhanced pepsin production, have been linked by a rare genetic relationship connected to the development of a stomach ulcer and gastric acid hypersecretion (Albaayit, 2016).

Perhaps the most prevalent bacterial infection worldwide is caused by the presence of *Helicobacter pylori* in the stomach and duodenum. *H. pylori* is gram negative,

microaerophilic rod-shaped bacterium. Typically, *H. pylori* infection is seen as the primary causative factor in the development of peptic ulcer pathology (Walker and Talley, 2014).

2.2 FACTORS REGULATING GASTRIC MUCOSAL INTEGRITY

Potentially precarious elements are constantly in contact with the stomach (Bell et al.,2018). Pepsin and hydrochloric acid, the alkaline duodenal contents' reflux that includes bile and pancreatic enzymes are endogenous substances that pose a threat to the stomach mucosa (Shahsavari and Parkman, 2022). Drugs particularly steroids, Aspirin, NSAIDs, and tobacco, cigarettes and alcohols consumptions are major exogenous mucosal excitations that might harm mucosae (Rusell, 2001; Cherkas and Zarkovic, 2018). The stomach's mucosal protection has the ability to defend itself against these elements. The gastrointestinal mucosal safety is thought to be maintained by gut defensive factors, which include mucus and bicarbonate released by surface epithelial cells, prostaglandins, sulfhydryl substances, and gastric mucosal blood flow (Oncel and Basson, 2022). Thus, the gut mucosa has established a barrier that separates the body from the luminal environment. The digestive tracts lining, or the epithelium, and secretions that affect the epithelial cells have barrier function. The gastrointestinal epithelium is made up entirely of cells that are covered by mucus. Mucus plays a crucial role in supporting the barrier function and the cut off pressures on the epithelium (Turner, 2009; Paone and Cani, 2020). Carbohydrates groups on mucin molecules react with bacteria, this interaction helps in preventing their aggregation and colonization. Mucus is expelled in feces and can serve as a bacterial trapping agent (Taherali, 2018; Paone and Cani, 2020). Furthermore, the antioxidant activity of mucus lessens the harm that bacteria and immune cells due to the mucosa (Turner, 2009; Giri et al., 2019). Mucus has a crucial constitutional role in the mucosal surface, helping to keep the pH of the surface close to neutral and acting as a physical barrier to luminal pepsin (Taherali, 2018).

Prostaglandins, leukotrienes, and thromboxanes are examples of bioactive lipids known as eicosanoids, which are crucial to the physiology of the stomach. Prostaglandins, in particular, frequently limit the release of gastric acid. The

inhibitory effect of PGE2 on acid output in humans, dogs, rats, mice, and monkeys has been demonstrated by earlier research (Zhang et al., 2022). Prostaglandins, whether endogenous or exogenous, activate the majority of the mucosal defense systems (Takeuchi and Amagase, 2018). The stomach mucosa's safety depends on the continuous production of prostacyclin and PGE2. Prostaglandins are important for maintaining mucosal blood flow and for stimulating the stomach's production of bicarbonate and mucus, reducing leukocytes dedicated to the endothelium, inhibiting acid release, mast cell activation and apoptosis, conserving or maintaining mucosal blood flow, and so averting ischemia (Zhang et al., 2022). Furthermore, it was discovered that prostaglandin F2 beta pretreatment decreased ethanol-related damage. 16, 16-dimethyl PGE2 completely prevents severe necrotic lesions, histologic alterations in the stomach mucus layer caused by 100% ethanol (Mikhailidis et al., 1990).

In the stomach mucosa, ethanol increases the generation of hydroxyl radicals, lipid peroxidation, and superoxide anion activity (Albano and Clot, 2017). Acute ethanol causes stomach mucosal cells to exhibit DNA damage, oxidative stress, elevated xanthine oxidase activity, malondialdehyde levels, and decreased total glutathione content (Boyacioglu et al.,2016). Another theory to explain ethanol-induced gastric mucosal injury is oxidative stress, which impairs mitochondrial energy metabolism and plays a major role in pathogenic ethanol-induced gastric mucosal injury (Qin et al., 2019).

The body typically maintains a balance between scavenging and free radical creation (Mccord,1993). Endogenous enzyme systems including superoxide dismutase, catalase, glutathione reductase, and coenzyme Q (Mirończuk-Chodakowska et al., 2018), as well as exogenous elements like vitamin C, vitamin E, selenium, and beta-carotene, are among the physiological defense systems that combat free radicals (Moussa et al., 2019). All of these molecules have a significant antioxidant role in the fight against oxidative stress because of their capacity to scavenge reactive oxygen species (ROS) using a redox-based mechanism or to transform ROS into stable and innocuous chemicals (Ighodaro et al., 2018). Increased activity of the hypothalamus pituitary adrenal axis and changes in the gastrointestinal tract are

physiological responses to stresses. The increase in the frequency of stomach ulcers is connected to this rise in adrenocortical activity (Filaretova et al., 2006). Stress ulcers are brought on by oxidative stress in the stomach, which also results in gastric ulcers (Das and Banerjee, 1993). Oxidative stress results in the activation of increased ROS production or a reduction in antioxidant defenses (Tandon et al., 2004). Oxidative stress has been influenced by the pathophysiology of stomach inflammation, ulcerogenesis, *H. pylori* infection, and lifestyle-related disorders such hypertension, atherosclerosis, ischemic heart disease, diabetes mellitus, and cancer (Tandon et al.,2004; Suzuki et al., 2011).

Free radicals can react with vital components in the cell to produce harmful effects. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxide (GPx) are examples of enzymatic and non-enzymatic antioxidant defense mechanisms that maintain physiological homeostasis by scavenging and controlling total ROS levels (Mirończuk-Chodakowska et al., 2018; Ighodaro et al., 2018).

2.3 NSAIDS' ASSOCIATED EFFECTS ON THE STOMACH MUCOSA

The stomach injury brought on by NSAID use is mediated by both cyclooxygenase-independent and dependent-inhibited pathways, the latter of which are mostly the consequence of local mode actions (Takeuchi, 2012). By suppressing several prostaglandin-mediated protective functions, cyclooxygenase inhibition has been demonstrated to increase the sensitivity of the stomach mucosa to NSAID-induced injury (Wallace, 2008). Prostaglandins, for example, lessen localized releases of reactive oxygen species (ROS) and neutrophil activation (Korbecki et al., 2013) . Mucosal microcirculation must produce prostacyclin in order to maintain a tonic inhibition of neutrophil adhesion (Fornai et al., 2011). Therefore, by inhibiting prostaglandin production, NSAIDs can tip the mucosal balance in favor of circulating neutrophil recruitment and endothelial adherence (Takeuchi, 2012). After adhering, neutrophils clog the microvasculature, resulting in a marked release of tissue-damaging factors such as leukotrienes and proteolytic enzymes, which heighten vascular tone, exacerbate tissue ischemia, increase ROS production (Fornai et al., 2011), and encourage intestinal matrix destruction, ultimately causing a severe

degree of focal tissue necrosis, especially when there is a low luminal pH (Witko-Sarsat et al., 2000). The stomach mucosal damage caused by NSAIDs is also facilitated by the cyclooxygenase-dependent reduction of bicarbonate secretion, as was previously mentioned (Takeuchi, 2012). In fact, the mucus gel layer's production of bicarbonate ions creates a pH gradient on the mucosal surface, acting as a first line of defense against luminal acid. Numerous investigations have revealed that the apical membranes of gastric surface epithelial cells exhibit bicarbonate/chloride ion exchangers and that prostaglandins produced by cyclooxygenase activate EP1 receptors to promote bicarbonate secretion (Blandizzi et al., 2009).

The majority of NSAIDs have a mildly acidic character, which explains why the gastric mucosa is injured locally by them even in the absence of cyclooxygenase (Hawkey, 2000). The hydrophobic surface barrier of the stomach may be weakened by the undissociated lipophilic form of acidic NSAIDs when there is gastric acidity. In experimental studies, this kind of impact plays a major role in NSAID-induced stomach damage, and it can last long after NSAID treatment is stopped (Fornai et al., 2011).

There is a constitutive expression of COX-1 and COX-2 in the stomach mucosa of rodents and humans (Zimmermann et al., 1998). Research using COX-1-knockout mice has shown that shown any indication of spontaneous gastric injury but NSAIDs can harm the gastric mucosa through COX-2-dependent pathways. Functions of COX isoforms in the stomach mucosa, demonstrated that COX-1-dependent prostaglandins are important in blood flow and mucus/bicarbonate secretion maintenance, while COX-2 provides localize mucosal protection against leucocyte endothelial adherence and promotes epithelial renewal (Jensen et al., 2018). While NSAIDs or the combined administration of COX-1 + COX-2 selective inhibitors caused gastric erosions, selective COX-1 or COX-2 inhibitors did not harm the stomach when evaluated alone, that NSAIDs may decrease gastrointestinal protection by concurrently blocking COX-1 and COX-2(Brzozowski et al.,2001).

2.4 ETHANOL INDUCES GASTRIC ULCERATION

Alcohol-induced stomach ulcers are among the most prevalent digestive system disorders, and their prevalence is rising (Zhang et al., 2016). Ethanol has the ability to directly dehydrate and penetrate the stomach lining mucosa, cytotoxic, direct cell membrane injury which makes it a possible risk factor for the formation of gastric ulcers. Ethanol has ability to solubilize the protecting mucus, thus removing the barrier mucus, and subsequently exposing the mucosa to the hydrolytic and proteolytic effects of pepsin and hydrochloric acid, which will ultimately result in gastric membrane damage (Burbige et., 1985; Birková et al., 2021). A higher level of oxidative stress in the tissues can be caused by ethanol through stimulating acid secretion, disrupting the vascular endothelium to decrease blood flow, and releasing superoxide anion. Since the coagulation processes are weakened, this impairment frequently may be associated with bleeding (Abdel-Kawi et al., 2022). Ethanol induced oxidative stress in gastric mucosa leads to reactive oxygen species (ROS) generation, that further leads to activation H+-K+-ATPases ion pumps induce high acidity gastric mucosal damage (Albano and Clot, 2017). Oxidative stress often leads to protein oxidation, lipid peroxidation and DNA damage in the gastric mucosa cell (Bhattacharyya et al., 2014). Ethanol induces mitogen-activated protein kinase II (MAPK-II), transcriptional factors for cyclooxygenase II (COX II), inducible nitric oxide synthase INOS leads to inflammatory response and gastric mucosal ulcer (Gomez et al., 2018).

2.5 CELLULAR MECHANISM(S) OF GASTRIC ULCER

The cyclooxygenase enzymes COX-1 and COX-2 have a major impact on mucosal defense. With the aid of COX-1, prostaglandins such as prostacyclin (PGI2) and PGE2 are generated and have the ability to protect the stomach mucosa by maintaining gastric blood flow, mucosal cell turnover, and mucus formation and secretion. COX-2 suppresses leukocyte adherence and maintains gastric blood flow. Enterochromaffin (EC) cells produce serotonin (5-HT), a neurotransmitter of the central nervous system (CNS), in the gastrointestinal tract (Dubois et al., 1998; Takeuchi et al., 2018). Intestinal 5-HT dysregulation has been linked to several

gastrointestinal disorders, including inflammatory bowel disease (IBD) (Khan, 2013; González et al., 2022).

The pro-inflammatory cytokine TNF has been linked to the pathophysiology of gastric ulcers. It inhibits constitutive nitric oxide synthase and can lead to the death of gastric parietal cells. This is significant because it may result in lower local concentrations of NO, a vasodilator that keeps the stomach mucosa's blood flow sufficient (Aziz et al., 2019). Additionally, neutrophils and macrophages have the ability to produce reactive oxygen species (ROS) and trigger the release of leukotrienes and proteolytic enzymes, both of which can damage tissue and hasten the establishment of stomach ulcers (Harijith et al., 2014).

Non-steroidal anti-inflammatory medication (NSAID) further damages stomach tissues by inhibiting both COX isoforms (Bindu et al., 2020). Alcohol use has the potential to directly affect stomach motility and metabolism. Alcohol causes gastric lesions in part by inhibiting antioxidant enzymes, which increases oxidative stress, modifies the NO system, and decreases blood flow to the stomach mucosa (Han et al., 2016). Reactive oxygen species (ROS) production is directly linked to stomach ulcers caused by ethanol, according to scientific reports. In the stomach, oxidative stress leads to an excess of reactive oxygen species (ROS), which damages cells (Bhattacharyya et al., 2014). Consequently, in order to preserve gastrointestinal homeostasis by scavenging reactive oxygen species (ROS), gastric cells show upregulation of a number of endogenous antioxidant enzymes, including catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) (Kumari et al., 2017). Additionally, alcohol-mediated reactive oxygen species (ROS) play a critical role in inflammation by promoting the production of pro-inflammatory cytokines like interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which worsen inflammation (Aziz et al., 2019).

2.6 MODERN MEDICINE IN MANAGEMENT OF GASTRIC ULCER

By scavenging free radicals produced by ulcerogenic substances, boosting mucosal defenses, or neutralizing stomach acid output, these drugs operate on particular receptors and activate specific pathways that support gastric ulcer therapy (Fornai et

al., 2011). The medications that are currently available to treat gastric ulcer conditions are proton pump inhibitors (Omeprazole, Pantoprazole, Lansoprazole, Rabeprazole, Esomeprazole)(Savarino et al.,2018), histamine H2-receptor antagonists(Cimetidine, Ranitidine, Famotidine, Nizatidine, Roxatidine, Lafutidine)(Singh et al.,2018), prostaglandin analogs, cyto-protective drugs (Rebamipide, Azulensulfonate, Teprenone, Polaprezinc, Sofalcone, Alginate sodium)(Morsy and El-Sheikh,2011), antibiotics(Amoxicillin, Tetracycline, Clarithromycin, Azithromycin) alone or in combination proton pump inhibitors and others inhibitors used to eradicate *H. pyl*ori(Safavi et al., 2016). When it comes to acid inhibition, proton pump inhibitors are more potent than histamine H2 receptor antagonists. To treat and prevent drug-induced peptic ulcers, current ulcer therapy includes proton pump inhibitors and *Helicobacter pylori* eradication through antibiotics for stomach ulcers that test positive for *Helicobacter pylori* (Dos Santos and Carvalho, 2015). The parietal cells' H+/K+ ATPase are specifically blocked by proton pump inhibitors (Savarino et al., 2018). While misoprostol is the most commonly used prostaglandin analog, its usage is restricted due to adverse effects that include diarrhea and cramping in the abdomen (Elati and Weeks, 2009). Bismuth and Sucralfate salts facilitate better mucosal healing and mucosal repair. Moreover, sucralfate works by lowering acid output and inhibiting the growth of *H. pylori* (Untersmayr, 2015). In conjunction with antibiotics, bismuth salts, which have a slight anti-*Helicobacter pylori* effect, are used in the treatment of stomach ulcers (Lambert and Midolo, 1997). However, there is ongoing debate about their effectiveness of aforesaid in preventing stomach ulcers in some specific predisposed populations. For instance, the normal dosage of the histamine H2 receptor antagonist only reduced the incidence of duodenal ulcers; it did not prevent gastric ulcers brought on by non-steroidal anti-inflammatory medicines (He et al., 2014). On the other hand, a study found that histamine H2 receptor antagonists showed comparable potency to proton pump inhibitors and was successful in preventing ulcers caused by low doses of aspirin (Chen et al., 2014). The efficacy of histamine H2 receptor antagonists were restricted to lowering the chance of ulcers brought on by an infection with *H. pylori.* Utilizing cyto-protective anti-ulcer medications to avoid stomach ulcers is another instance, albeit its effectiveness is still debatable (Morsy

and El-Sheikh, 2011). The kind of predisposing risk factor will therefore determine whether to use these traditional anti-ulcer medications to avoid gastric ulcers. The evaluation's risk variables are such as age, the existence of cardiovascular conditions, the use of excessive doses or numerous non-steroidal combined uses of low-dose aspirin and other anti-platelet medications, anti-inflammatory medications medicines and corticosteroids (Valkhoff et al., 2012). It is advised to switch from nonselective to selective cyclooxygenase inhibitors when three or more risk factors are present, as this indicates a significant risk. Apart from their efficacy these chemical anti-ulcer agents have several side effects in the patients. Examples of side effects include nausea, vomiting, constipation, diarrhea, and stomach discomfort from proton pump inhibitors (omeprazole, lansoprazole), and gynecomastia and decreased libido from H2 receptor antagonists (cimetidine) (Piper, 1995; Borrelli and Izzo, 2000). Prolonged usage of these medications may result in a number of negative side effects, including hepatotoxicity, thrombocytopenia, gynecomastia, nephrotoxicity, and impotence (Sheen and Triadafilopoulos, 2011).

2.7 HERBAL AND MEDICINAL PLANTS IN THE ANTI-ULCER MANAGEMENTS

Due to its efficiency, low cost, and no or less side effects, herbal treatments are becoming more and more popular now days as a cost-effective solution for managing and preventing stomach ulcers. Since herbal remedies are thought to be less harmful and less toxic when used to treat ulcers, a great deal of research is done to find powerful antiulcer compounds derived from plants. Traditionally, medicinal plants have been used all throughout the world to cure ulcers. There are numerous scientific reports and studies pertaining to anti-ulcer activities of herbal plants in various models.

The anti-ulcer activity of methanol extracts obtained from the leaves of *Intsia bijuga*, *Cynometra ramiflora*, *Tamarindus indica*, *Cassia javanica*, *C. fistula*, *Bauhini purpurea*, *Senna spectabilis*, *S. siamea*, and *Saraca thaipingensis* were assessed using HCl-ethanol as the ulcerogenic agent (0.2 mL per 20 g body weight of 0.3 M HCl/60% ethanol solution). All extracts exhibited inhibitory activity, with *I. bijuga*,

T. indica, *S. spectabilis*, and *S. thaipingensis* demonstrating more than 50% inhibition. Significantly, *S. thaipingensis* demonstrated the most pronounced activity, achieving an inhibition rate of 80%, which was akin to the positive control sucralfate, exhibiting an inhibition rate of 83%. Further partitioning of *S. spectabilis* and *S. thaipingensis* into hexane, ethyl acetate, and aqueous fractions revealed that the aqueous and ethyl acetate fractions of *S. spectabilis* showed significant increases in activity, with values of 79.3% and 71.7%, respectively. Similarly, the hexane and ethyl acetate fractions of *S. thaipingensis* exhibited higher activity, each with a value of 63%, compared to its aqueous fraction (Paguigan et al., 2014). The antiulcer activity of an alcoholic extract derived from *Cynodon dactylon* was evaluated in Wistar albino rats at dosage levels of 200, 400, and 600 mg/kg body weight. The extract at doses of 400 mg/kg and 600 mg/kg demonstrated significant ($p < 0.001$) antiulcer activity when compared to the standard drug ranitidine. This alcoholic extract mitigated ulcer formation by reducing both output volume and total acidity (Patil et al., 2003). The antiulcerogenic activity of hydroalcoholic extract obtained from *Glycyrrhiza glabra* L. (HEGG) at dose levels of 50, 100, 150 mg/kg, and 200 mg/kg was assessed using various ulcer induction methods including HCl/Ethanol (0.2 mL of a 0.3 M HCl/60% Ethanol solution), Indomethacin (60 mg/kg p.o dissolved in 5% NaCO3), and Ethanol (0.2 ml/animal, p.o). Omeprazole was utilized as a positive control (reference drug) at doses of 30 mg/kg. The HEGG (50–200 mg/kg) demonstrated a significant reduction in ulcer index induced by HCl/Ethanol. Furthermore, the extract effectively inhibited the formation of gastric lesions induced by ethanol. Notably, the extract at a dose of 200 mg/kg exhibited higher potency compared to omeprazole (30 mg/kg) (Jalilzadeh-Amin, 2015). The anti-ulcerative properties of methanolic extracts derived from *Helianthemum lippii* (MHL) were investigated using an ethanol-induced ulcer model in Wistar albino rats. The treatments consisted of 1 mL of normal saline (negative control), standard ranitidine (50 mg/kg), MHL at doses of 250 mg/kg, and MHL at doses of 500 mg/kg. Ulcer induction was achieved by orally administering absolute ethanol (99%) (1 mL per animal). Animals treated with the methanol extract of MHL at doses of 250 mg/kg and 500 mg/kg exhibited significant ($p < 0.05$) inhibition of ulceration by 48.78% and 76.82%, respectively, while ranitidine demonstrated a 69.78% inhibition of

ulceration (Alsabri et al., 2013). The hydroalcoholic extract obtained from the leaves of *Annona muricata* L. (HEAM) was evaluated for its anti-ulcerative properties using absolute ethanol, acidified ethanol, and indomethacin as gastric inducers in male and female albino mice (*Mus musculus*). Saline (0.1 mL/kg p.o) served as a negative control, while Omeprazole (30 mg/kg p.o) served as a positive control. HEAM extract doses of 50, 100, 200, and 400 mg/kg significantly reduced the lesion area by 92.89%, 94.13%, 97.79%, and 96.55%, respectively, when gastric lesions were induced by absolute ethanol (0.2 mL per animal). HEAM at doses of 200 and 400 mg/kg exhibited greater effectiveness than the standard drug (omeprazole). Omeprazole (30 mg/kg p.o.) significantly reduced gastric lesion areas by 95.79% compared to the control ($p < 0.001$). In groups where gastric lesions were induced by acidified ethanol (0.3 M HCl in 70% ethanol solution) and pretreated with HEAM at doses of 50, 100, 200, and 400 mg/kg, reductions in lesions of 47.69%, 76.23%, 80.20%, and 93.22% were observed, respectively. At a dose of 400 mg/kg, HEAM was as effective as the standard drug (omeprazole), which reduced the ulcer area by 93.22%. Omeprazole (30 mg/kg) significantly reduced gastric lesion areas by 84.56% ($p < 0.001$) compared to the control. When gastric lesions were induced by the non-steroidal anti-inflammatory drug indomethacin (10 mg/kg), HEAM at a dose of 200 mg/kg exhibited the most effectiveness, reducing the incidence of ulcers by 94.13%. HEAM at 400 mg/kg also significantly decreased the incidence of ulcers by 91.67%. Omeprazole (30 mg/kg) reduced lesion rates by 96.82% compared with the control group (Bento et al., 2018).

The dried roots of *Arctium lappa* L. underwent trituration and extraction using ethanol 96% through percolation. This ethanolic extract was evaluated for its anti-ulcerative properties using Bethanechol (2.5 mg/kg, s.c.), Pentagastrin (100 μg/kg, s.c.) (NSAID), and Histamine (20 mg/kg, s.c.) as ulcer inducers in female Wistar rats (*Rattus norvegicus*). Negative control consisted of vehicle (water 1 mL/kg i.d.), while positive control was Ranitidine (50 mg/kg i.d.). The extract at a dose of 1000 μg/kg prevented the increase in gastric secretion in pylorus-ligated rats stimulated by bethanechol, histamine, or pentagastrin. Moreover, the extract at a dose of 10 mg/kg i.d. reduced the acidity of gastric secretion induced by bethanechol, histamine, or

pentagastrin (da Silva et al., 2018). The gastroprotective potential of aqueous extracts derived from *Artemisia absinthium* L. aerial parts, obtained through hot aqueous extraction (HAE) and room temperature aqueous extraction (RTAE), was assessed in female Wistar rats using an acute gastric ulcer model induced by ethanol p.a. Negative control consisted of vehicle (water 10 mL per kg), while positive control was omeprazole (20 mg/kg). The extracts at concentrations of 3%, 10%, and 30% exhibited gastroprotective effects in the submucosal layer, leading to a reduction in lesion area by 64.68%, 53.71%, and 90.04%, respectively (Boeing et al., 2023). In this investigation, female Wistar rats (*Rattus norvegicus*) received treatment with aqueous leaf extract of *Casearia sylvestris* Sw. (AEC) at doses of 3, 30, or 300 mg/kg before induction of gastric ulcers using ethanol (1 mL), piroxicam (100 mg/kg), or 80% acetic acid. Negative control consisted of vehicle (1 mL saline, orally), while positive control was omeprazole (40 mg/kg). AEC at doses of 30 or 300 mg/kg exhibited preventive effects against ulcers induced by ethanol and piroxicam. Additionally, at a dose of 300 mg/kg, AEC accelerated the healing process of acetic acid-induced ulcers by 48%, as evidenced by ultrasonography, which revealed a reduction in wall thickness and extent of edema in ulcerous lesions promoted by the extract (de Oliveira et al., 2022). The gastroprotective potential of *Cinnamomum glanduliferum* (Wall) Meissn leaf extract was evaluated at doses of 250, 500, and 1000 mg/kg in an ethanol-induced (1 mL) non-ulcerative gastritis model using male Wistar albino rats (R. norvegicus). Normal saline (1 mL, orally) and indomethacin (10 mg/kg) served as negative and positive controls, respectively. The leaf extract at doses of 250, 500, and 1000 mg/kg significantly ($p < 0.05$) reduced paw volume to 94%, 82%, and 69%, respectively, and significantly decreased COX-2 activity to 73.8, 50.7, and 21.4 nmol/min/mL, respectively. Moreover, the *C. glanduliferum* extract demonstrated a significant ($p < 0.05$) modulatory effect on ethanol-induced gastritis in rats, evidenced by a reduction in NO levels to 32, 37, and 41 µM nitrate/g, and a significant ($p < 0.05$) inhibition of lipid peroxidation through reduction of MDA concentration to 1.15, 1.11, and 1.04 nmol/g (Azab et al., 2017).

The dried powdered leaves of *Emex spinosa* (L.) Campd. were subjected to methanol (MeOH) extraction using a maceration protocol. *Rattus norvegicus* (Wistar albino) rats received doses of 250 and 500 mg/kg body weight of the extract, while the standard group was administered omeprazole at a dose of 20 mg/kg orally, with 100% ethanol (1 mL/100 g) inducing gastric ulcers. The leaf extracts, at doses of 250 mg/kg and 500 mg/kg, decreased acidity and provided protection to the stomach mucosa wall (Ajaib et al., 2022). In another study, the methanolic extract of *Melissa officinalis* (MO) leaves was investigated for its anti-ulcerative properties using indomethacin (NSAID) at a dose of 48 mg/kg orally to induce gastric ulceration in male Wistar rats. Vehicle (1 mL saline orally) and ranitidine (25 mg/kg) were employed as negative and positive controls, respectively. No significant difference in the ulcer index was observed between the MO extract (150 and 300 mg/kg) and ranitidine-treated rats (Saberi et al., 2016). The investigation focused on the antiulcer activity of the methanolic extract derived from *Phyllanthus niruri* L. leaves, administered orally at doses of 100, 200, and 400 mg/kg. This activity was assessed against ethanol-acid-induced gastric mucosal injury in Swiss albino rats, with omeprazole (20 mg/kg, administered orally) serving as the reference standard. The results indicated that the extract, at doses of 100, 200, and 400 mg/kg, significantly ($p < 0.05$) reduced gastric erosion induced by ethanol-acid by 69.59%, 74.32%, and 80.40%, respectively, in all experimental groups compared to the control group (Mostofa et al., 2017). The study employed the dried hydroalcoholic extract derived from *Tagetes erecta* L. flowers (DHETE) to investigate its intestinal anti-inflammatory properties using some male Swiss mice (*M. musculus*) model of ulcerative colitis (UC). Experimental UC was induced by supplementing the drinking water with 5% dextran sulfate sodium (DSS), and the effects of DHETE at doses ranging from 30 to 300 mg/kg were examined. The negative control group received a vehicle solution consisting of 10 mL per kg distilled water orally administered with 1% Tween 80, while the positive control group was treated with 5-ASA at a dose of 100 mg/kg orally. Mice in the colitis group treated with *T. erecta* L. (300 mg/kg, administered once daily for 9 days) exhibited an 11% reduction in body weight. Additionally, the extract led to a 16% decrease in colon shortening compared to the vehicle-treated colitis group, along with a 26% reduction in IL-6 levels and a 22%

increase in glutathione (GSH) availability (Meurer et al., 2019). The aqueous fraction (FrAq) derived from *Terminalia catappa* L. leaves was employed to elucidate its antiulcer mechanism in male Wistar rats, utilizing 1 mL of absolute ethanol to induce gastric ulcers. Negative control comprised vehicle (10 mL/kg saline), while Lansoprazole (30 mg/kg) served as positive control. At a dosage of 25 mg/kg, the extract notably diminished the number of ethanol-induced ulcerative lesions, while activating mucosal protective factors such as the nitric oxide (NO) pathway and endogenous prostaglandins. Oral administration of the extracts for 7 and 14 days resulted in a significant ($p < 0.05$) reduction in lesion area (80% and 37%, respectively) compared to the negative control group (Silva et al., 2015).

The effects of the ethanolic bark extract of *Amphipterygium adstringens* Schide ex Schlecht (AaEE) were investigated in a model of colitis induced by 4% Dextran sulfate sodium (DSS) in albino mice (Bagg Albino). Following treatment with AaEE at a dose of 200 mg/kg, there was a significant reduction in ulcer area, alleviation of edema and adhesion, decreased infiltration of inflammatory cells, and attenuation of damage to the colon's mucosa. The extract exhibited a diverse array of activities, including the reduction of oxidative stress, suppression of inflammation, modulation of multiple signal transduction pathways, and induction of apoptosis (Rodriguez-Canales et al., 2016). The effects of the aqueous-methanol extract (AFE) derived from *Anneslea fragrans* Wall. leaves were examined in a dextran sodium sulfate (DSS)-induced (2.5%, w/v) model of ulcerative colitis (UC) using Kunming mice (*Mus musculus* Km.). A negative control comprising distilled water with DSS (2.5%, w/v) and a positive control involving Sulfasalazine (450 mg/kg) were utilized. Administration of *A. fragrans* Wall. extract at doses of 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg resulted in elevated levels of IL4 and IL10, accompanied by reduced levels of IL6, IL1β, and TNF-α. Additionally, the extract significantly inhibited the phosphorylation of target proteins within the NF-κB and MAPK signaling pathways in colon tissue (Deng et al., 2021). Mangiferin, sourced from *Belamcanda chinensis* rhizomes, was investigated for its impact on colon tissues in 2,4,6-trinitrobenzensulfonic acid (TNBS)-induced colitis in Wistar rats (*Rattus norvegicus*). A vehicle comprising distilled water and TNBS solution (0.3 mL/kg)

served as the negative control. Pre-administration of mangiferin demonstrated a protective effect, mitigating the damage induced by TNBS acid. Mangiferin doses of 30 and 100 mg/kg notably decreased both macroscopic and microscopic damage scores as well as the level of malondialdehyde (MDA) in colon tissues. Specifically, the 100 mg/kg dose of mangiferin exhibited a reduction in tumor necrosis factor alpha (TNF-α) and interleukin-17 (IL-17) concentrations, along with an increase in superoxide dismutase (SOD) activity within colon tissues (Szandruk et al., 2018). The effectiveness of *Berberis lycium* Royle fruit extract (BLFE) in alleviating 3.5% dextran sulfate sodium (DSS)-induced colitis was investigated in albino mice (Bagg Albino). Negative control groups received vehicle (distilled water and 3.5% DSS solution), while positive control groups were administered 5-aminosalicylic acid (ASA) at a dose of 250 mg/kg. BLFE treatment significantly improved the survival rate of animals, Disease Activity Index (DAI) score, colon length, and structural damage in mice exposed to DSS. BLFE appeared to modulate intestinal epithelial cell proliferation (PCNA) and apoptosis (Bcl2/Bax), suggesting its role in maintaining intestinal integrity (Sharma et al., 2020). Acute ulcerative colitis (UC) induced by 4% dextran sodium sulfate (DSS) was established in adult male C57BL/6 mice. The DSS-induced mice received treatment with either *Canna x generalis* rhizome ethanol extract (CGE) at doses of 100 and 200 mg/kg or sulfasalazine (SAS) at a dose of 200 mg/kg for 7 days. Negative control groups received vehicles (distilled water and 0.5% CMC Na), while positive control groups were administered sulfasalazine (200 mg/kg). The findings indicated that DSS exacerbated the cytoplasmic expression of the apoptotic execution marker "caspase-3," primarily observed within the degenerated crypts. Treatment with either SAS or CGE at a dose of 100 mg/kg notably down-regulated the immunohistochemical (IHC) expression of caspase-3, as evidenced by a reduction in the percentage of caspase-3 positively stained areas compared to the DSS-induced colitis group. However, CGE at a dose of 200 mg/kg exhibited a significant decrease in caspase-3 expression compared to either SAS or CGE at a dose of 100 mg/kg (Mahmoud et al., 2021).

Ethanol extract of *Centella asiatica* (L.) Urb. (CA) was investigated for its effects on ulcerative colitis induced by dextran sulfate sodium (DSS) in a murine colitis model

using male albino mice (*B. albino*). A negative control (vehicle: 3% distilled water) and a positive control (5-aminosalicylic acid [ASA]: 400 mg/kg) were employed. The macroscopic damage scores of ASA and both CA-treated groups (200 and 400 mg/kg) were significantly reduced ($p < 0.01$). Additionally, the disease activity index (DAI) score of the model group was notably higher than that of the control group, but it was significantly decreased in the ASA and CA groups. These findings indicate that CA effectively ameliorated DSS-induced colitis. Administration of CA at doses of 100, 200, and 400 mg/kg improved intestinal motility and modulated the gut microbiome of mice experiencing discomfort by reducing the abundance of colitis-associated genera such as Helicobacter, Jeotgalicoccus, and Staphylococcus, thus altering the community and enhancing α-diversity (Li et al., 2021).

The efficacy of Evodiamine (EVO) in alleviating 2.5% dextran sodium sulfate (DSS)-induced ulcerative colitis (UC) was assessed in male C57BL/6 mice. Evodiamine (EVO) is a principal alkaloidal compound derived from *Evodia rutaecarpa*. Treatment with EVO significantly reduced disease activity index (DAI) scores compared to the DSS group. Administration of EVO at doses of 20, 40, and 80 mg/kg showed notable prevention of colonic shortening. Symptoms such as mucosal erosion, hyperemia, neutrophil infiltration, loss of cryptal glands, and goblet cell depletion observed in the DSS group were not evident in the EVO-treated groups. These findings suggest that EVO possesses protective effects against DSS-induced UC in mice (Shen et al., 2019). Ulcerative colitis was induced in 10-week-old C57BL/6 male mice by providing purified drinking water containing 4% (w/v) dextran sulfate sodium (DSS), and the potential attenuation of ulcerative colitis by koreanaside A (KA) was examined. Koreanaside A, a lignan isolated from the flowers of *Forsythia koreana*, was administered orally. Distilled water containing 4% DSS served as the vehicle, while 5-aminosalicylic acid (ASA) (75 mg/kg) was used as the positive control. Treatment with KA (5 or 20 mg/kg) significantly reduced disease activity index (DAI) values. Similar suppressive effects were observed with 5-ASA administration. In mice with DSS-induced colitis, KA alleviated colitis symptoms by suppressing inflammatory cell infiltration, restoring expression of tight junction (TJ)- and epithelial–mesenchymal transition (EMT)-

related proteins, and inactivating AP-1, NF-κB, and STAT1/3 (Kim et al.,2019). The administration of kolaviron a compound derived from *Garcinia kola* and sulfasalazine (control drug) reduced the levels of hydrogen peroxide and lipid peroxidation, increased antioxidant status, and lessened the negative impact of DSS induction on colon architecture, thereby ameliorating colitis caused by DSS (Farombi et al., 2013). The study aimed to investigate the potential inhibitory effect of Grape seed polyphenols (GSP) on Ulcerative colitis (UC) induced by 2.5% dextran sulfate sodium (DSS). GSP was administered at different doses (500 and 750 mg/kg body weight per day). Mice treated with both high and low doses of GSP exhibited significantly lower total Disease Activity Index (DAI) scores compared to those in the DSS group, with GSP-H showing superior efficacy over GSP-L. Treatment with GSP at different doses also significantly mitigated colon shortening compared to the DSS group. The extract alleviated mucosal injury, inflammatory infiltration, bloody stools, diarrhea, and weight loss, potentially reducing intestinal epithelial cell (IEC) apoptosis by down regulating mRNA expression of the inflammatory cytokines IL-6, IL-1β, and TNF-α, as well as phosphorylation of STAT3. Furthermore, there was a significant ($p < 0.05$) decrease in the levels of cytokines IL-6, IL-1β, and TNF-α in the GSP-treated groups compared to the control group, indicating the potential therapeutic effects of GSP on UC (Wang et al., 2020).

Gymnema sylvestre (GS) leaf extract at doses of 50, 100, and 200 mg/kg/day was investigated for its preventive effects against acetic acid (AA)-induced ulcerative colitis in Wistar albino rats (*Rattus norvegicus*). Distilled water containing 200µL of 4% AA solution served as the vehicle control, while Mesalazamine (300 mg/kg) was used as the positive control. The GS extract significantly ($p < 0.05$) preserved mucosal integrity, attenuated oxidative and inflammatory responses, increased colonic weights compared to the control group, exhibited antioxidant properties, and reduced acetic acid levels in the colon. Furthermore, the extract markedly increased superoxide dismutase (SOD) activity compared to the control group. It also significantly ($p < 0.05$) reduced levels of pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 compared to animals in the vehicle group (Aleisa et al.,2014). The effects of methanol extracts from *Ilex kudingcha* C.J. Tseng (KME) were investigated on 3%

dextran sulfate sodium (DSS)-induced ulcerative colitis (UC) in male C57BL/6J mice. KME administration significantly attenuated DSS-induced body weight loss and colon length shortening, and reduced the colon weight-to-length ratio. Additionally, KME treatment increased glutathione (GSH) levels and decreased myeloperoxidase (MPO) and malondialdehyde (MDA) levels in colon tissue. KME mitigated edema, mucosal damage, and loss of crypts induced by DSS. Administration of KME at doses of 50 and 200 mg/kg respectively reduced colonic MDA levels to 0.79±0.07 and 0.63±0.07 nmol/mg protein, and downregulated the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Song et al., 2013). The effects of polysaccharide extracted from the fruits of *Morinda citrifolia* L. (Noni-PLS) were investigated against intestinal damage in ulcerative colitis (UC) induced by acetic acid (6%) in male Swiss mice. Noni-PLS was administered intraperitoneally at doses of 0.1, 0.3, and 3.0 mg/kg. Treatment with Noni-PLS resulted in a reduction of acetic acid-induced intestinal damage, as evidenced by decreases in macroscopic and microscopic scores as well as in the wet weight of the colon. Furthermore, Noni-PLS treatment led to reductions in myeloperoxidase (MPO) activity, levels of glutathione (GSH), malondialdehyde (MDA), nitrate/nitrite (NO3/NO2), pro-inflammatory cytokines, and cyclooxygenase-2 (COX-2) expression (Batista et al., 2020).

The efficacy of *Olea europaea* Hoffmannsegg leaves extract standardized with 25% hydroxytyrosol (OLES-25%HYT) was investigated in the treatment of 4% acetic acid-induced ulcerative colitis in male albino rats. OLES-25%HYT, administered at a dose of 150 mg/kg diluted in 1 ml saline, demonstrated effectiveness in reducing mortality rate and disease activity index (DAI). Moreover, it mitigated oxidative stress and inflammation in colon tissue, as evidenced by a significant decrease in colon levels of malondialdehyde (MDA), myeloperoxidase (MPO), and nitric oxide (NO), along with a significant increase in levels of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). Furthermore, OLES-25%HYT down regulated the expression of pro-inflammatory cytokines (Abd Elmaksoud et al., 2021). The potential therapeutic effects of Asian dandelion roots (*Taraxacum officinale*) extract on ulcerative colitis (UC) were investigated using a murine model.

Acute ulceration was induced by administering 2% dextran sulfate sodium (DSS) to female C57BL/6 mice. Treatment with dandelion root extract at concentrations of 3 mg/mL and 6 mg/mL resulted in a reduction in DSS-induced apoptosis, with the higher concentration demonstrating a more pronounced effect (15.79% and 10.28% reduction, respectively). Additionally, administration of dandelion root extract mitigated progressive acute injury, as evidenced by decreases in body weight loss, disease index severity scores and colon length during DSS treatment. Furthermore, dandelion root extract attenuated inflammatory conditions and oxidative stress in the colons of mice with DSS-induced colitis (Ding et al., 2018). The therapeutic potential of *Terminalia chebula* (TCE) extract was investigated in Inbred Charles-Foster (CF) strain albino rats weighing between 150–170 g with 10% acetic acid (AA)-induced colitis. TCE, administered at a dose of 600 mg/kg, exhibited healing effects against AA-induced colonic damage score and weight loss. Intra-colonic administration of AA led to heightened colonic mucosal damage, inflammation; mucus/bloody diarrhea, and stool frequency, accompanied by reduced body weight. However, both TCE and sulfasalazine (SS, used as a positive control) treatments reversed these effects. Treatment with TCE led to a reduction in fecal blood and mucus, significant decreases in stool frequency by 19.3% and 11.5%, and a tendency to increase or increased body weight by 2.7% and 9.7%, respectively, compared to the AA group. Furthermore, SS-treated rats exhibited a decrease in fecal blood and mucus, significant decreases in stool frequency by 21.8% and 23.9%, and an increase in body weight by 11.7% and 12.1%, respectively, compared to the AA group (Gautam et al., 2013). The study aimed to assess the impacts of a mouthwash derived from the decoction of *Nicotiana tabacum* (L.) leaves. Participants were randomly assigned to receive either tobacco or placebo mouthwash (10 mL, 3 times a day) for 5 days. Results revealed that in the tobacco group, the ulcer pain score decreased by 79.2% and 93.8% on days 3 and 5, respectively, while ulcer size decreased by 69.1% and 92.2% on the same respective days. These reductions were significantly greater compared to the placebo group ($P < 0.01$) (Vaziri et al., 2016).

The efficacy of licorice (*Glycyrrhiza glabra*) in eradicating Helicobacter pylori was investigated in patients diagnosed with dyspepsia, including both peptic ulcer disease

(PUD) and non-ulcer dyspepsia (NUD), and compared to a standard triple regimen containing clarithromycin (500 mg BID), amoxicillin (1 gr qd), and omeprazole (20 mg BID) as the control group. The study demonstrated that adding a low dose of licorice (D-Reglis 380 mg BID) to the standard triple treatment led to a more effective eradication of *H. pylori*, particularly in patients with PUD (Hajiaghamohammadi et al., 2016). The impact of ingestible nanoparticles derived from ginger in treating colitis-associated cancer was investigated using female FVB/NJ mice (6–8 weeks old). Colitis was induced by supplementing with 1.5% (w/v) dextran sodium sulfate (DSS). GDNPs 2 (0.3 mg per mouse) were administered orally, with untreated animals used as controls. GDNPs 2 mitigated acute colitis, promoted intestinal regeneration, and prevented the onset of chronic colitis and colitis-associated cancer (CAC). The oral intake of GDNPs 2 resulted in enhanced survival and proliferation of intestinal epithelial cells (IECs) and decreased levels of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β), alongside increased levels of anti-inflammatory cytokines (IL-10 and IL-22) in colitis models. These findings indicate that GDNPs 2 exhibits potential in mitigating detrimental factors while fostering a reparative effect (Zhang et al., 2016). The study explored the impact of a buccoadhesive paste (FBP) formulated from Fenugreek seed extract (*Trigonella foenum graecum* L.) in the treatment of Recurrent aphthous stomatitis (RAS). Dexamethasone mouthwash was used as the positive control. Beginning on the fourth day of treatment, patients receiving FBP demonstrated notable enhancements in lesion size and pain severity. By the sixth day of the intervention, there was a significant reduction ($p < 0.05$) in both erythema level and ulcer size (Ansari et al., 2022). The molecular mechanism involved gastric ulcer modulations by herbal plants were given in the table no.1.

Table 1: **Antiulcer activity of various plants and their molecular targets**

Source	Family	Molecular targets	References
Abelmoschus esculentus (L.) Moench	Malvaceae	TBARS, PGE_2	Yokota et al., 1996
Acacia nilotica Linn	Fabaceae		Bansal & Goel, 2012
Aegle marmelos (L.) Corrêa	Rutaceae	SOD, CAT, GSH, LPO	Das & Roy, 2012
Alhagi maurorum Medik	Fabaceae	GSH, MDA	Shaker et al., 2010
Allium ampeloprasum L.	Amaryllidaceae		Adão et al., 2011
Allium ascalonicum L.	Amaryllidaceae	antibacterial activity	Adeniyi & Aniyam, 2004
Allium sativum L.	Amaryllidaceae	TNF-α, MDA, PGE_2, GSH	El-Ashmawy et al., 2016
Allium sativum L.	Amaryllidaceae	IL-10, IL-1 β, TNF-α, CAT, SOD	Keiss et al., 2003; Tope et al., 2014
Allium sativum L.	Amaryllidaceae	LTB4, mucus MPO, TNF-α, IL-1β, $cPLA_2$, COX_2	Choi et al., 2014
Allium sativum L.	Amaryllidaceae	Nrf2, HO-1, NQO1	Kim et al., 2014
Allium sativum L.	Amaryllidaceae	SOD, GPX, CAT, MDA	Khosla et al., 2004
Anethum graveolens L.	Apiaceae		Hosseinzadeh et al., 2002
Angelica sinensis (Oliv.) Diels	Apiaceae	LPO, EGF, H^+-K^+ ATPase	Sgarbossa et al., 2015; Umre et al., 2018
Annona muricata L.	Annonaceae	CAT, SOD, GSH, NO, PGE_2, glycogen, Hsp70, Bax, MDA	Moghadamtousi et al., 2014
Annona squamosal L.	Annonaceae	H+K+-ATPase activity, gastrin	Yadav et al., 2011
Apium graveolens L.	Apiaceae		Baananou et al., 2013
Apium graveolens L.	Apiaceae	GWM, NP-SH, MDA	Al-Howiriny et al., 2010
Aronia melanocarpa (Michx.) Elliott	Rosaceae	PGE_2, MDA	Valcheva-Kuzmanova et al., 2005
Aronia melanocarpa (Michx.) Elliott	Rosaceae		Matsumoto et al., 2004
Artocarpus obtusus	Moraceae	GSH, NP-SH, NO,	Sidahmed et al.,

F.M. Jarrett		MDA, Bax, HSP70, COX-2	2013
Asparagus africanus var. puberulus (Baker) Sebsebe	Asparagaceae		Nwafor et al., 2000
Asparagus racemosus Wild.	Asparagaceae	CAT, SOD, ascorbic acid, LPO	Bhatnagar et al., 2006
Asparagus racemosus Wild.	Asparagaceae	Mucus	Sairam et al., 2002
Azadirachta indica A. Juss.	Meliaceae	K^+- H^+ ATPase, LPO, MMP	Chattopadhyay et al., 2004, Yadav et al., 2017
Benincasa hispida Cogn.	Cucurbitaceae		Grover et al., 2001
Benincasa hispida Cogn.	Cucurbitaceae	MDA, SOD, VitC	Shetty et al., 2008
Bombax ceiba L.	Malvaceae	Gastric secretion	Hussain et al., 2015
Brassica oleracea L.	Brassicaceae	Collagen fibers proliferation in mucus layer	Carvalho et al., 2011
Brassica oleracea L,	Brassicaceae	Mucus	Lemos et al., 2011
Butea monosperma (Lam.) Taub.	Leguminosae	Inflammatory mediators	Ammar et al., 2011; Banji et al., 2011
Calotropis procera (Aiton) Dryand.	Apocynaceae	5-LOX, Peptic activity	Sen et al., 1998
Calophyllum brasiliense Cambess	Clusiaceae	MDA, CAT, GSH	Lemos et al., 2012; Silva et al., 2019
Camellia sinensis (L.) Kuntze	Theaceae	LPO	Maity et al., 1995; Nair et al., 2010
Capsicum annuum L.	Solanaceae		Montero et al., 2015
Capsicum annuum L.	Solanaceae	CGRP, CAT, SOD, caspase-3, TNF-α, MDA	Li et al., 2012
Carica papaya L.	Caricaceae	Gastrointestinal motility	Ezike et al., 2009
Carica papaya L.	Caricaceae	GSH, MDA	Oloyede et al., 2015
Carica papaya L.	Caricaceae	GSH, mucus production	Pinto et al., 2015; Okewumi and Oyeyemi, 2012
Cenostigma	Fabaceae	NO, CAT, KATP	Viana et al., 2013

macrophyllum Tul.		channel opening	
Centella asiatica (L.) Urb.	Apiaceae	iNOS, NO	Cheng et al., 2004, Gohil et al., 2010
Cichorium intybus L.	Asteraceae		Krylova et al., 2015
Citrullus lanatus (Thunb.) Matsum. & Nakai	Cucurbitaceae	CAT, SOD, LPO	Sharma et al., 2014
Citrus aurantium L.	Rutaceae	GSH, GR, GPx, NO, MDA, MPO, PGE_2	Bonamin et al., 2014
Citrus aurantium L.	Rutaceae	VEGF, gastric mucus	Moraes et al., 2013
Citrus aurantium L.	Rutaceae	VEGF, gastric mucus	Polo et al., 2012
Citrus limon (L.) Osbeck	Rutaceae	Antibacterial activity	Filocamo et al., 2015
Citrus lemon (L.) Osbeck	Rutaceae	Mucus, HSP-70, VIP, PGE_2, antibacterial activity	Rozza et al., 2011
Citrus trifoliata L.	Rutaceae.	Mucus secretion, antibacterial activity	Lee et al., 2009
Cocos nucifera L.	Arecaceae		Nneli & Woyike, 2008
Crataegus L.	Rosaceae		Tadić et al., 2008
Cucurbita pepo L.	Cucurbitaceae		Sarkar & Guha, 2008
Cucumis maderaspatanus L.	Cucurbitaceae	MDA, TNF-α, mucin, CAT, NO, PGE_2	Gomathy et al., 2015
Curcuma amada Roxb.	Zingiberaceae	H+K+ATPase activity, LPO	Siddaraju & Dharmesh, 2007
Curcuma longa L.	Zingiberaceae	IL-8, IL-1 β, TNF-α, COX-2, GSH	Akbik et al., 2014; Koosirirat et al., 2010
Cydonia oblonga Mill.	Rosaceae	MPO	Hamauzu et al., 2006
Cynara scolymus L.	Asteraceae	Mucus, MDA	Nassar et al., 2013
Cynara scolymus L.	Asteraceae	Mucus	Ishida et al., 2010
Daucus carota L.	Apiaceae	Mucus, GSH, Acid	Chandra et al., 2015
Entada africana Guill. & Perr.	Fabaceae.	Infiltration of neutrophil, eosinophil and lymphocytes	Obidike & Emeje, 2011
Eruca vesicaria (L.)	Brassicaceae	MDA	Alqasoumi et al.,

Cav.			2009
Eugenia astringens Cambess.	Myrtaceae		Meyre-Silval et al., 2009
Ficus racemosa L.	Moraceae	H+K+ATPase activity, mucus, CAT, GSH, LPO	Rao et al., 2008
Fragaria ananassa Duchesne ex Decne. & Naudin	Rosaceae	CAT, SOD, MDA	Alvarez-Suarez et al., 2011
Garcinia humilis (Vahl) C.D. Adams	Clusiaceae		Niero et al., 2012
Garcinia gummi-gutta (L.) N. Robson	Clusiaceae		Mahendran et al., 2002
Garcinia indica (Thouars) Choisy	Clusiaceae	ROS formation	Yamaguchi et al., 1999
Glycyrrhiza glabra L.	Leguminosae	TNF- α, NO, IL-6, CAT, SOD, GSH	Fatima et al., 2008, Aslam et al., 2015
Gynostemma pentaphyllum (Thunb.) Makino	Cucurbitaceae		Hesse et al., 2007
Gynostemma pentaphyllum (Thunb.) Makino	Cucurbitaceae	Mucus, hexosamine	Rujjanawate et al., 2004
Hymenaea stigonocarpa Mart. ex Hayne	Fabaceae	GSH, MPO, delayed gastric emptying	Rodrigues et al., 2012
Lagenaria breviflora (Benth.) Roberty	Cucurbitaceae	ROS formation	Onasanwo et al., 2011
Livistona chinensis (Jacq.) R.Br. ex Mart.	Arecaceae		Kadry et al., 2009
Livistona decora (W. Bull) Dowe	Arecaceae		Kadry et al., 2009
Malus domestica (Suckow) Borkh.	Rosaceae	GSH	Paturi et al., 2014
Malus domestica Suckow) Borkh.	Rosaceae	COX-2, HB-EGF, MDA	D'Argenio et al., 2008
Malus domestica Suckow) Borkh.	Rosaceae	MPO	Hamauzu et al., 2006
Mammea Americana L.	Clusiaceae		Toma et al., 2005
Mangifera indica L.	Anacardiaceae	NO, TBARS, GSH	Priya et al., 2011
Mangifera indica L.	Anacardiaceae	NO, SH compounds	Lima et al., 2006
Mangifera indica L.	Anacardiaceae		Severi et al., 2009
Maytenus gonoclada Mart.	Celastraceae	CAT, GSH, SOD, MDA	da Silva et al., 2015

Melia azedarach L.	Meliaceae	Acid secretion induced by elevated TLP	Moursi& Al-Khatib, 1984
Momordica charantia L.	Cucurbitaceae	Collagen content. Mucin, SOD	Alam et al., 2009
Momordica charantia L.	Cucurbitaceae		Gürbüz et al., 2000
Momordica charantia L.	Cucurbitaceae		Dengiz & Gürsan, 2005
Momordica cochinchinensis Spreng.	Cucurbitaceae	MPO, cPLA2, 5-LOX	Lim et al., 2014
Momordica cochinchinensis Spreng.	Cucurbitaceae	CGRP, cPLA2, 5-LOX, LTB4, MPO, IL-1β, TNFα	Kang et al., 2009
Momordica cochinchinensis Spreng.	Cucurbitaceae	Gastrin, cPLA2, COX-2, 5-LOX	Choi et al., 2012
Momordica cochinchinensis Spreng.	Cucurbitaceae	VEGF	Kang et al., 2009
Momordica cochinchinensis Spreng.	Cucurbitaceae	Stimulation of CGRP and somatostatin receptors	Jung et al., 2013
Momordica dioica Roxb. ex Willd.	Cucurbitaceae	H+K+ATPase, CAT, LPO	Vijayakumar et al., 2011
Moringa pterygosperma Gaertn.	Moringaceae	Mucin secretion	Akhtar et al., 1995
Musa paradisiaca L.	Musaceae	5-Hydroxytryptamine	Goel et al., 1984
Musa paradisiaca L.	Musaceae		Lewis et al., 1999
Musa paradisiaca L.	Musaceae	Mucosal protein, SA, Hex, NO in mucosal tissue, NO in serum	Prabha et al., 2011
Musa paradisiaca L.	Musaceae	LPO	Goel et al., 2001
Musa paradisiaca L.	Musaceae		Pannangpetch et al., 2001
Musa paradisiaca L.	Musaceae		Lewis et al., 1999
Musa paradisiaca L.	Musaceae	Stimulation of gastric mucosal growth	Best et al., 1984
Muntingia calabura L.	Muntingiaceae	Mucus, PGE_2, NO, SOD, GSH, CAT, MDA	Zakaria et al., 2016
Muntingia calabura L.	Muntingiaceae	LOX, NO, XO	Balan et al.,

			2015; Buhian et al., 2016
Myristica malabarica Lam.	Myristicaceae	PGE_2, VEGF, vWF VIII, EGF, endostatin	Maity et al., 2008
Myristica malabarica Lam.	Myristicaceae	Non-protein thiol, PGE_2, mucin, TBARS, protein carbonyl	Banerjee et al., 2008
Myristica malabarica Lam.	Myristicaceae	Non-protein thiol, LPO, protein carbonyl	Banerjee et al., 2007
Myrtus communis L.	Myrtaceae.		Sumbul et al., 2010
Ocimum tenuiflorum L	Lamiaceae	NF-KB, IL-6, LPO, MDA	Chaiyana et al., 2019; Singh and Chaudhuri, 2018
Opuntia ficus-indica (L.) Mill.	Cactaceae		Galati et al., 2003
Panax ginseng C.A. Mey	Araliaceae	IL-1, iNOS, KC, LPO, TNF- α, NO, COX-2, NF-KB	Bae et al., 2014; Baek et al., 2016, Han et al., 2018
Parkia platycephala Benth.	Fabaceae	NO, CAT	Fernandes et al., 2010
Parkia speciosa Hassk.	Fabaceae	HSP70, GSH, SOD, BAX, MDA	Al Batran et al., 2013
Phanera vahlii (Wight & Arn.) Benth.	Fabaceae	Peptic activity	Akhtar et al., 1995
Phoenix dactylifera L.	Arecaceae	mucin, HA, gastrin	Al-Qarawi et al., 2005
Phyllanthus emblica L.	Phyllanthaceae	PGE2, e-NOS/i-NOS ratio	Chatterjee et al., 2012
Phyllanthus emblica L.	Phyllanthaceae	ILs, PGE2, TNF- α	Chatterjee et al., 2010; Hasan et al., 2016
Phyllanthus emblica L.	Phyllanthaceae	MDA, protein carbonyl, total thiol, TNF-α, IL-1β, IL-10	Chatterjee et al., 2011
Phyllanthus emblica L.	Phyllanthaceae	CAT, GSH, MDA	Pakrashi et al., 2003
Phyllanthus emblica L.	Phyllanthaceae	Nonprotein sulfhydryl	Al-Rehaily et al., 2002
Phyllanthus emblica L.	Phyllanthaceae	Mucus, LPO, CAT, Acid, pepsin	Sairam et al., 2002
Phyllanthus emblica L.	Phyllanthaceae	Gastric mucus, hexosamine	Bandyopadhyay, 2000
Picrorhiza kurroa	Plantaginaceae	COX-2, VEGF, EGF	Kumar et al.,

Royle ex Benth.			2017; Soni and Grover, 2019
Pithecellobium dulce (Roxb.) Benth.	Fabaceae	Mucin, PGE2, pepsin, MPO, H+K+ATPase activity	Megala & Geetha, 2012
Pleurolobus gangeticus (L.) J.St.-Hil. ex H.Ohashi & K.Ohashi	Fabaceae.		Dharmani et al., 2005
Psidium guajava L.	Myrtaceae		Livingston & Sundar, 2012
Pseudocydonia sinensis (Thouin) C.K. Schneid.	Rosaceae	MPO	Hamauzu et al., 2006
Punica granatum L.	Rosaceae	SOD, CAT, GSH, GPx, TLP, TP	Ajaikumar et al., 2005
Punica granatum L.	Rosaceae	Mucus	Alam et al., 2010
Punica granatum L.	Rosaceae	MDA, NO	Lai et al., 2009
Raphanus raphanistrum subsp. sativus (L.) Domin	Brassicaceae		Devaraj et al., 2011
Rheum emodi Wall.	Polygonaceae	Antibacterial activity	Ibrahim et al., 2006
Rhizophora mangle L.	Rhizophoraceae	PGE2, EGF, COX, NO, MPO, TNF- α, ILs	de Faria et al., 2012; Melchor et al., 2001
Rubus coreanus Miq.	Rosaceae	GPx, SOD, TBARS	Nam et al., 2006
Rubus coreanus Miq.	Rosaceae	CAT, SOD, GPx, MMP-2, TNF-α, IL-1β	Kim et al., 2011
Rubus imperialis Cham. & Schltdl.	Rosaceae		Berté et al., 2014
Rubus fruticosus Lour.	Rosaceae,	NF-κB driven transcription, NF-κB translocation, release of IL-8, SOD, CAT, Trolox equivalents, release of CINC-1	Sangiovanni et al., 2013
Rubus idaeus L.	Rosaceae,	NF-κB driven transcription, NF-κB translocation, release of IL-8, SOD, CAT, Trolox equivalents, release of CINC-1	Sangiovanni et al., 2013
Sapindus mukorossi Gaertn.	Sapindaceae	Antibacterial activity	Ibrahim et al., 2006

Sapindus saponaria L.	Sapindaceae.		Albiero et al., 2002
Schisandra chinensis (Turcz.) Baill.	Schisandraceae.	Pepsin activity, ATP	Hernandez et al., 1988
Solanum aethiopicum L.	Solanaceae		Chioma et al., 2011
Solanum lycopersicum L.	Solanaceae	MDA, MPO, SOD, GSH	Boyacioglu et al., 2016
Solanum nigrum L.	Solanaceae	Acid, pepsin, Gastrin, H+K+ ATPase activity	Jainu & Devi, 2006
Solanum tuberosum L.	Solanaceae	H+ K+-ATPase activity, antibacterial activity	Chandrashekar & Dharmesh, 2016
Stryphnodendron adstringens (Mart.) Coville	Fabaceae		Martins et al., 2002
Stryphnodendron rotundifolium Mart.	Leguminosae	PG, NO, LPO	da Costa et al., 2012; Oliveira et al., 2014
Syzygium cumini (L.) Skeels	Myrtaceae	GSH, MPO	El-Shenawy, 2009
Terminalia chebula Retz.	Combretaceae	H^+-K^+ ATPase	Raju et al., 2009; Sharma et al., 2011
Tetradium ruticarpum (A.Juss.) T.G.Hartley	Rutaceae	Antimicrobial activity	Hamasaki et al., 2000
Trichosanthes cucumerina L.	Cucurbitaceae	Mucus, acid, HA	Arawwawala et al., 2010
Trichosanthes kirilowii Maxim.	Cucurbitaceae.		Takano et al., 1990
Vaccinium oxycoccos L.	Ericaceae	Antibacterial activity	Burger et al., 2002
Vasconcellea pubescens A.DC.	Caricaceae	Mucus depletion	Mello et al., 2008
Wilbrandia ebracteata Cogn.	Cucurbitaceae.		Coelho et al., 2009
Wilbrandia ebracteata Cogn.	Cucurbitaceae.		Gonzalez et al., 2002
Withania somnifera (L.) Dunal	Solanaceae	CAT, SOD, ascorbic acid, LPO	Bhatnagar et al., 2005
Xylocarpus granatum J. Koenig	Meliaceae		Lakshmi et al., 2010
Xylocarpus moluccensis M. Roem.	Meliaceae		Lakshmi et al., 2014
Zaleya pentandra (L.) C. Jeffrey	Aizoaceae		Akhtar & Ahmad, 1995

Zea mays L.	Poaceae		Tovey et al., 2005
Zhizhu decoction *(Aurantii fructus* and *Atractylodes macrocephala)*	Rutaceae and Asteraceae	VEGF, MDA, Hsp70, NOS, Gastric emptying	He et al., 2013
Zingiber officinale Roscoe	Zingiberaceae	LPO, NF-$_K$B, H^+-K^+ ATPase	Pan et al., 2008
Ziziphus lotus Lam.	Rhamnaceae		Wahida et al., 2007

CHAPTER 3

MATERIAL AND METHODS

3.1 Plant materials

A. *Mallotus roxburghianus* Mull.Arg.

Systematic position:

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Malpighiales

Family: Euphorbiaceae

Genus: Mallotus

Species: *Mallotus roxburghianus*

Fig. 3.1

Deciduous shrub with whitish-brown stem, long petiole pubescent serrate rough leaves. The plant species is native to tropical forest area of south East Asian regions such as Assam, Bangladesh, China South-Central, East Himalaya, Myanmar, Nepal. In India it mostly reported from Assam, Meghalaya and Mizoram. In Mizoram mainly found particularly in the tropical evergreen forests and mixed bamboo forests. In Mizo language it is locally known as "Zawngtenawhlung". Traditionally used in blister, diabetes mellitus, foodborne diseases, hepatomegaly, hypertension, jaundice, liver diseases, and ulcer in North East India (Fig. 3.1). The ethanol extract of *M. roxburghianus* has been reported for its anti-diabetic and anti-oxidant activity (Lalhlenmawia et al., 2007; Roy et al., 2016). Phytochemicals such as

Methoxybenzoic acid, rhamnopyranoside, betulinic acid and bergenin isolated and identified from the leaves of *M. roxburghianus* (Rana et al., 2005).

B. *Garcinia lanceifolia* Roxb.

Systematic position:

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Malpighiales

Family: Clusiaceae

Genus: Garcinia

Species: *Garcinia lanceifolia*

Fig. 3.2

Evergreen moderately small size tree species, bark can be grey, reddish, or pale gray; branches are frequently opposing, terete, and glabrous; latex is typically thick, sticky, and yellow in the bark. Simple leaves with an acute to obtuse base that are lanceolate-ovate to oblong-oblong and opposite or occasionally ternate. Fruit in the persistent Berries with flesh, either smooth or sulcate, and persistent sepals. Ovoid, oblong seeds with a creamy white flesh covering them. *G. lanceifolia* is an important endemic medicinal plant of NE-India mainly found in Assam and Mizoram, frequently known as "Rupahi-thekera" (Assamese), "Pelte" (Mizo), "Rupohi tekera" (Mising), belonging to the family Clusiaceae. Fruit is edible and locally sold (Fig. 3.2). The plant is well for its ethnomedicinal values for stomachic & diuretic in Mizoram, pain reliever and hypoglycemic agents in Assam (Singh et al., 2002; Baruah 2007; Bora et al., 2014). The plant known harbor rich source of bioactive compounds such as phenolics,

tannins, saponins, flavonoids & terpenoids, alkaloids with potential anti-oxidative, anti-bacterial, anti-inflammatory and antifungal properties (Chowdhury & Handique, 2012; Policegoudra et al., 2012; Bora et al., 2014).

3.2 Identification of plant materials and Extraction of plant materials:

Fresh *M. roxburghianus* leaves were gathered from Ngopa village, Champhai district (Saitual district), Mizoram (22. 520 °N, 92. 890 °E) with permit during March - April 2015. The plant was identified and authenticated by a taxonomist and a voucher sample (MZU/ZOO/2015/Mar-1999) was assigned and preserved in the Department of Zoology, Mizoram University, Aizawl, Mizoram. Fresh fruits of *G. lanceifolia* were collected from Thingdawl village (24. 1663 °N; 92. 6947 °E; 673m), Kolasib district, Mizoram, in May 2021. The plant was locally and botanically identified and validated as per Flora of Mizoram (Singh *et al*, 2002) and a voucher sample (1540 dated 21-09-2023) was assigned and preserved in the Mizoram University Herbarium (Singh *et al*, 2002).

3.2.1 Soxhlet extraction of *M. roxburghianus* leaf sample

The grounded air-dried powdered of *M. roxburghianus* leaf samples 50 g and 250 mL methanol (90%) were taken into the soxhlet apparatus extracted for 8 hours at 55-85°C. A heating mantle was used to reflux the mixture hence temperature was adjusted accordingly. The extract solution was allowed to cool at room temperature following the completion of the extraction time. After that, it was filtered using a filter paper cone (Whatman no. 1). The solvent extracts were concentrated by rotary vacuum evaporator at 40 °C, and extracted methanolc leaf sample of *M. roxburghianus* (MRME) was air dried and then stored at 4 °C for further use.

3.2.2 Cold maceration extraction of *G. lanceifolia* fruit sample

The coarse dried grounded fruit rind powder of *G. lanceifolia* (100 g) was put in the 1 litre conical flask, and poured 500 ml of methanol (90%). The flask was closed with aluminium foil, extracted for four days at room temperature. The flask with sample was intermittently shaked during extraction period. The methanolic fruit

extract (GLME) was filtrated through Whatman filter paper (No. 1) and then concentrated at 40 °C using a rotary evaporator. The fruit extract was air dried and stored in the refrigerator (4ºC) for further use.

3.3 LC–MS/MS analysis of *M. roxburghianus* methanol leaf extract

LC-MS/MS analysis of the methanol fraction of MR was carried out using an Agilent 6400 series triple quadrupole B.07.00 (B7022.6 SP1, Agilent, USA) equipped with a degasser, binary gradient pump, column thermostat, auto sampler, UV detector and an ESI source. For the chromatographic separation, a C18 reversephase analytical column was employed (Zorbax 300A, SB-C18, 150 2.1 mm i.d., 3.5 lm particle); the work temperature was 48 °C. The elution gradient comprised of mobile phase A (water, 5 mM ammonium formate and 0.1% formic acid) and mobile phase B (methanol, 5 mM ammonium formate and 0.1% formic acid). The gradient program with the following proportions of solvent B was applied t (min), % B: (0, 40), (20, 90), (23.99, 90), (24, 40), (29, 40). The solvent flow rate was maintained at 0.5 mL/min and injection volume was settled as 4 mL. Subsequent to several combinations of trials, a gradient of methanol (5 mM ammonium formate and 0.1% formic acid) and water (5 mM ammonium formate and 0.1 % formic acid) system was used for rich ionization and the separation of the molecules. Tandem mass spectrometry was used for fragmented ion stability. The following parameters were used: the ions were monitored in full scan in positive and negative mode, nitrogen was used for both nebulizer and collision gas for electrospray ionization with positive and negative ion polarity the declustering potential 100 eV, entrance potential 10 eV, collision energy 35 eV, and collision cell exit potential 10 eV were set, the drying temperature to 350 °C, the nebulizer pressure to 40 psi, and the drying gas flow to 15 L/min. The maximum accumulation time was 50 ms, the scan speed was 26,000 m/z/s and the fragmentation time was 30ms. The fragmentation profile was obtained from the selection of a pattern ion and monitored in MS2 mode. The target ions monitored were 169 for gallic acid; 301 for quercetin; 353 for chlorogenic acid; 463 for hyperoside and 609 for rutin. The limit of detection and limit of quantitation of the method with good linearity (R2 > 0.95) were determined and

calibration curve (0.05–50 mg/ mL) was established from six measurements using standards (Hadrich et al., 2014).

3.4 HR-LCMS of methanol extract of *G. lanceifolia*

The separation of *G. lanceifolia* fruit rind methanol extract chemical constituents were revealed with a Q-Exactive Plus Biopharma-High Resolution Orbitrap Liquid Chromatograph Mass Spectrometer, Thermo Scientific Instrument. Data Acquisition and Data Processing was carried out using Thermo Scientific Xcalibur, Version 4.2.28.14 and Compound Discoverer 2.1 SP1 through Windows XP. The chromatographic separation was performed using Hypersil Gold 3micron 100 x 2.1 MM (Thermo Scientific) analytical column used as a stationary phase at 40°C as temperature. The mobile phase consisted of methanol (solvent B) and 0.1% formic acid in milliq water (0.1 mL formic acid /100 mL water – Solvent A). The flow rate was kept at 0.3 mL/min. Mass spectra data were recorded on an ionization mode (both positive and negative) for a mass range of m/z 100-1500 and runtime is 0-30 min. For the identifications of phytochemical compounds by mzCloud library were used as the main tools at Indian Institute Technology (IIT), Bombay.

3.5 Laboratory animals and ethical permit

Colony bred male and female Wistar albino rats (170–200 g, 3 months old) were maintained under controlled standard animal house conditions (temperature: 25–27°C; photoperiod: 12 h natural light and 12 h dark; humidity: 80–90%) with access to food and water ad libitum (standard pellet diet; Pranav Agro Industries, Maharashtra, India) at animal care facility at the Department of Zoology, Mizoram University, Aizawl, Mizoram, India. This study was carried out following approval from the committee on the ethics of animal experiments of the Mizoram University animal ethical committee (MZUAEC), Mizoram University, Aizawl, Mizoram, India (permit No. 1: MZUIAEC/29/03/2016-17/09/ZOO/SLS and permit No. 2: MZU/IAEC/2020/09) on the use and care of experimental animals.

3.6(I) Acute toxicity of MRME and GLME

A total of 18 rats (9 males and 9 females) were equally divided into 3 groups that administered with the vehicle (0.5% CMC), and 1000 and 3000 mg/kg of MRME and for GLME vehicle (0.5% CMC) and 1000, 2000 and 3000 mg/kg of GLME to determine a safe dose as per guidelines 425 (OECD. 2001). The animals were monitored for 48 h after the administration of the MRME and GLME for the onset of clinical or toxicological symptoms and further observations were continued up to 15 days all the rats were examined for general behavioral abnormalities, signs of toxicity, and mortality. Mortality and behavioral alterations were noted (Cheesbrough, 2009). During treatment, daily observations were taken of the rat's body weight, food and water intake, abnormal behaviors, and severe toxicity symptoms. Every week, the weight was recorded. All rats were anaesthetized at the end of the treatment, and blood samples were collected immediately for hematological and biochemical analysis. A sample of the tissues used for histological analysis, and the organs (liver, spleen, kidney) were collected and weighed to calculate organ coefficients.

(II)Sub-Acute toxicity of GLME:

Sub-acute toxicity study (28-day repeated oral toxicity study) was carried out according to OECD 407 guidelines. Male and female rats (130-200g) were divided into four groups (3 females and 2 males in each): Group I: Control where the animals were given no any treatment. Group II: 50 mg/kg b.wt. of methanol extract of Garcinia lanceifolia in distilled water for 28 consecutive days. Group III: 100 mg/kg b.wt. of Garcinia lanceifolia in distilled water for 28 consecutive days. Group IV: 200 mg/kg b.wt. of Garcinia lanceifolia in distilled water for 28 consecutive days

All the groups of rats were observed twice daily for mortality and morbidity till the completion of the experiment). During treatment, daily observations were taken of the rat's body weight, food and water intake, abnormal behaviors, and severe toxicity symptoms. Every week, the weight was recorded. All rats were anaesthetized at the end of the treatment, and blood samples were collected immediately for

hematological and biochemical analysis. A sample of the tissues used for histological analysis, and the organs (liver, spleen, kidney) were collected and weighed to calculate organ coefficients.

3.7 Experimental Design of Anti-ulcer activity

3.7.1 *M. roxburghianus*

A total 45 rats were kept for fasting for 24 h and randomly divided into 9 groups (in each group n=5 rats with no male/female preference). Groups were divided as follows; Group I: Normal control group without any pretreatment and no ethanol adminitration, Group II: Ethanol ulcerated group (administration of 5 ml/kg ethanol without any pretreatment), Group III: Pretreatment with Omeprazole (Standard anti-ulcer drug; 20mg/kg) and Ethanol (5 ml/kg) after 1 h, Group IV: Pretreatment with MRME (5mg/kg) and Ethanol (5 ml/kg) administration after 1 h, Group V: Pretreatment with MRME (10mg/kg) and Ethanol (5 ml/kg) administration after 1 h, Group VI: Pretreatment with MRME (20mg/kg) and Ethanol (5 ml/kg) administration after 1 h, Group VII: Pretreatment with MRME (50mg/kg) and Ethanol administration (5 ml/kg) administration after 1 h, Group VIII: Pretreatment with MRME (100mg/kg) and Ethanol administration (5 ml/kg) administration after 1 h, Group IX: Pretreatment with MRME (200mg/kg) and Ethanol administration (5 ml/kg) administration after 1 h, according to the method described by (Morimoto *et al*, 1994) with slight modifications. One hour later, all the rats were euthanized through an overdose of ketamine, photos of the stomachs were taken (Al-Obaidi, et al., 2014), and Image J software was used to measure the gastric lesions (Bethesda, MD, USA) (Fig. 3.3).

3.7.2 *G. lanceifolia*

A total 45 rats were kept for fasting for 24 h and randomly divided into 6 groups (in each group n=5 rats). Groups were divided as follows; Group I: Normal control group without any pretreatment and no ethanol administration, Group II: Ethanol ulcerated control group (administration of 5 ml/kg ethanol without any pretreatment), Group III: Positive control group i.e., pretreatment with Omeprazole (Standard anti-

ulcer drug; 20 mg/kg) and Ethanol (5 ml/kg) after 1 h, Group IV: Pretreatment with GLME (200 mg/kg) and Ethanol (5 ml/kg) administration after 1 h, Group V: Pretreatment with GLME (400 mg/kg) and Ethanol (5 ml/kg) administration after 1 h, Group VI: Pretreatment with MRME (600 mg/kg) and Ethanol (5 ml/kg) administration after 1 h, Group according to the method described by (Morimoto *et al*, 1994) with slight modifications. One hour later, all the rats were euthanized through an overdose of ketamine, photos of the stomachs were taken (Al-Obaidi, et al., 2014), and Image J software was used to measure the gastric lesions (Bethesda, MD, USA).

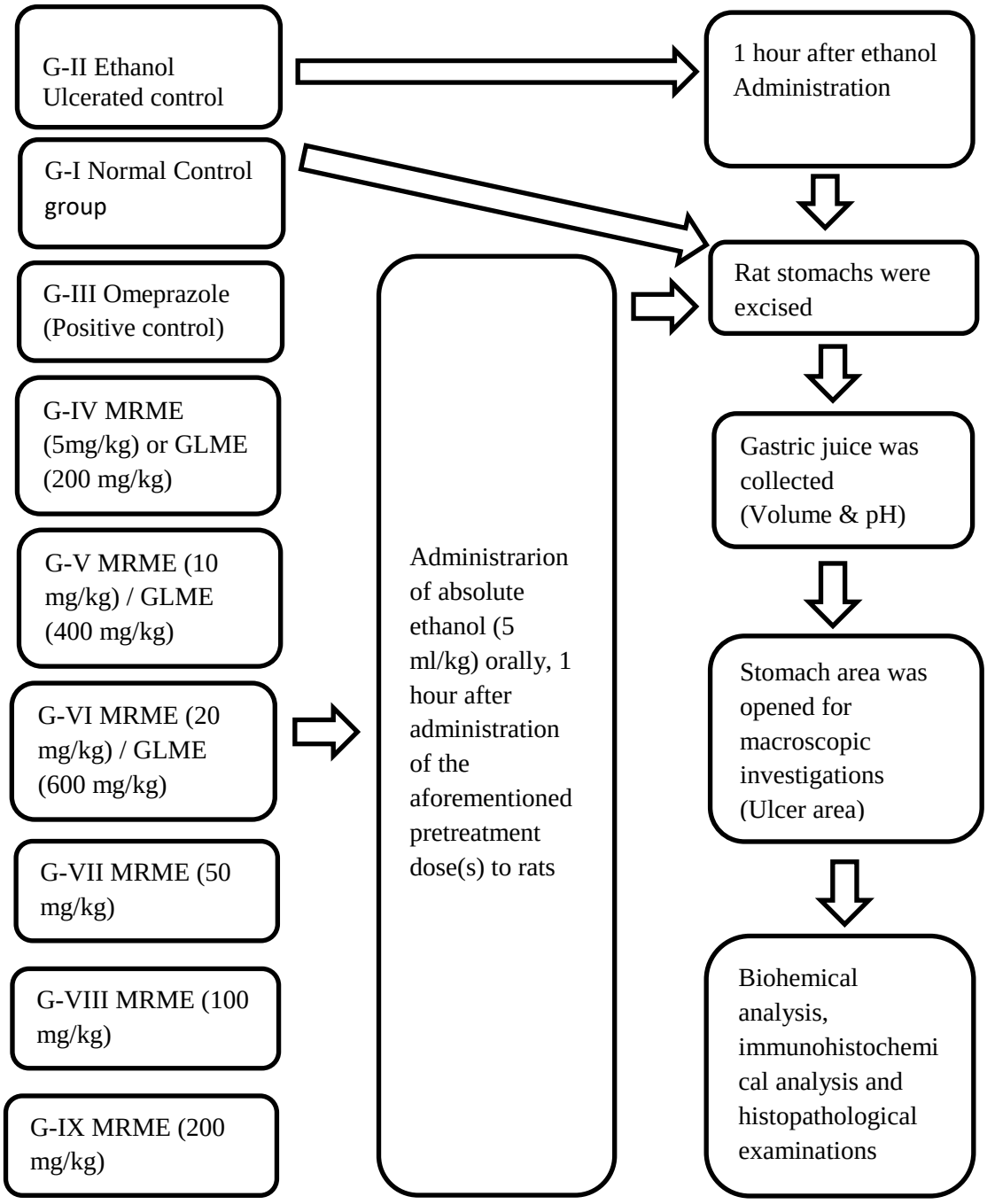


Fig. 3.1 schematic illustration of experimental design (n=5 rats for each group) (MRME-M. roxburghianus methanolic extract, GLME-G. lancefolia methanolic

3.8 Measurement of gastric juice pH, acidity, and gastric wall mucus (GWM)

Measurement of pH: All animal groups' stomach contents were removed, and they were then centrifuged, collected supernatant gastric fluids were measured for their pH by digital pH meter.

A bigger curvature incision was made in the stomach, and the contents were subsequently emptied, gathered, and centrifuged at 1000g for 10 min at 4C. The total acidity was determined and expressed as mEq/L. A round conical flask (50 mL) was filled with 1 mL water and 1 mL centrifuged gastric content. Subsequently, a few drops of phenolphthalein indicator were added to the flask, and the mixture was titrated with NaOH (0.01 N) until a pink color formed in the solution. It was measured how much titre (NaOH 0.01 N) was needed. In the following formula, the total acidity is evaluated. Total acidity =n × 0.01 × 36.45 × 1000. In the aforesaid formula, where n is the volume of NaOH used, 0.01 is denoted NaOH normality, and 36.45 is indicated as NaOH Mol. The weight element in the equation is 1000 (given in liters) (Evans, 2009). GWM content was measured by using Alcian blue at 580 nm (Piper et al., 1970).

3.9 Ulcer index and preventive index

The gastric contents were drained and washed with saline, and each stomach was examined under a magnifying lens to determine the ulcer index. The severity of ulcers was scored as follows: score 0: normal-colored stomach; score 0.5: red coloration; score 1: spot ulcer; score 1.5: hemorrhagic streak; score 2: deep ulcers; and score 3: perforation.

Ulcer score was calculated as the mean of ulcers in each group (total number of ulcers divided by rat's number, n = 6), and ulcer index (UI) was then calculated by multiplying ulcer score 100. The preventive index was calculated as: {[UI of ulcerated group - UI of treated group] 100)/UI of ulcerated group} (Bardi et al., 2011).

3.10 Hematoxylin and eosin (H& E) staining and PAS staining

Sections of the stomach samples gathered were prepared for histopathological analysis. The specimens were preserved in formalin with a 10% phosphate buffer for a minimum of 48 hours. After that, the tissues were cut with a rotating microtome. (Leica, model RM2125 RTS), samples were dehydrated with 70%, 80%, 90%, and 100% alcohol, cleared in three grades of xylene, then fixed in melted wax. After the blocks solidified, they were divided into 5µm thick sections using a rotary microtome, submerged in a water bath, and then incubated for 30 minutes at 60°C. The 5µm thick sections thick were subsequently cleaned in 3 grades of alcohol (100%, 90%, and 70%). The sections were then stained with hematoxyllin & eosin. Mountants (DPX) were used to create permanent mounts on degreased glass slides. For histological changes in gastric tissues (edema, lining, necrosis, perforations, bleeding, and congestion) observed with a regular light microscope (Leica DM 2500) with a digital camera (model-DFC 450C) (Leica Microsystems, Wetzlar, Germany) and imaged (Bancroft and Gamble, 2008). Mucosal glycoproteins in the gastric tissue sections were evaluated by Periodic acid Schiff staining (McManus, 1948).

3.11 Quantification of mucin

The mucosal glycoproteins stained by PAS were quantified using ImageJ software (Jensen, 2013) with the following setting options (Rangan & Tesch, 2007): (1) choosing a suitable microscopic field for histopathology analysis wherein (a) 10–20 fields were identified using a predetermined selection procedure, (b) five or six dots circumferentially around the gastric sections were placed and examined fields perpendicular to the dot as noncontiguous random fields and (c) GU lesions are usually assessed at a magnification of 100; (2) calibration of measurement units, (3) assessment of area stained (Area fraction) and (4) setting of thresholds for a particular color.

3.12 Computer assisted cell counting of mast cells and eosinophils

For the determination of the eosinophil and mast cell counts in the stomach wall, five tissue sections were made for each stomach (H&E, PAS) and in each slide, ten fields

were counted. The total number of eosinophilic cells in GM and submucosa and the intact and active degranulated mast cells obtained in ten fields of five different sections was noted and mean was determined. Computer assisted cell counting (eosinophils and mast cells) was performed under a light microscope at a magnification of 40 with an optical micrometer using ImageJ cell counter. - jar software (Rangan & Tesch, 2007). Counting was done within the region of interest and used to identify the number of cells labeled per unit area. The results were expressed in terms of total number of cells per mm2 area (mean ± standard error mean**).**

3.13 Lipid peroxidation and antioxidant enzymes activity

The gastric tissue was homogenized with ice cold Tris EDTA suspension buffer (10% w/v, 50 mmol, pH 7.8) using a homogenizer. The homogenates were filtered and centrifuged for 30 min at 10,000g at 4 ^{o}C and the supernatants were frozen at 8 ^{0}C in aliquots until used for biochemical assays. The protein content of the supernatant was determined using the Lowry method (Lowry et al., 1951). Lipid peroxidation was assessed by measuring the thiobarbituric acid reactive substances and quantified as malondialdehyde equivalents (MDA, nmol/mg protein) concentrations by using 1, 1, 3, 3-tetramethoxypropane as the standard (Ohkawa et al., 1979). Superoxide dismutase (SOD, U/mg protein) activity was measured at 560 nm by the nitro blue tetrazolium reduction method (Asada et al., 1974). Glutathione S-transferase (GST, U/mg protein) activity was computed at 340 nm using 1-chloro-2, 4-dinitrobenzene as a substrate (Habig et al., 1974).

3.13.1 Measurement of lipid peroxidation

The Malondialdehyde (MDA) level of the stomach was determined according to the principle previously described with minor modifications (Ohkawa et al., 1979). In brief, 75 µl of stomach lysates were mixed with the same amount of 10% Trichloroacetic acid (TCA). Freshly prepared 0.2% Thiobarbituric acid was then added in a ratio of 1:2 and then boiled for 45minutes. The solutions were allowed to cool down and the optical densities were measured against the blank at 532 nm and

the concentration of MDA was expressed as nM/mg protein. A mixture devoid of stomach protein lysate served as blank.

3.13.2 Superoxide dismutase (SOD) assay

The previously described method (Asada et al., 1974) was adopted for the superoxide dismutase assay. In brief, a mixture containing 50 µl of 10% tissue homogenate, 600 µl of 52 mM sodium pyrophosphate buffer (pH 8.3), 50 µl of 186 µM Phenazine methosulphate (PMS), 150 µl of 300 µM Nitroblue tetrazolium(NBT). The reaction was started by the addition of 100 µl of 750µMml NADH and incubated at 30 °C. The reaction was terminated after 90 seconds incubation by the addition of 500 µl glacial acetic acid. Two ml of n-butanol was added, vortexed and allowed to stand for 10 minutes. The mixture was centrifuged at 10000 × g for 10minutes at room temperature and the supernatant was collected. Color intensity was measured at 560 nm and the concentration of SOD was expressed as units (U)/mg protein. A mixture devoid of tissue homogenate was taken as blank.

3.13.3 Glutathione reduced (GSH) assay

An earlier described principle and method was adopted for Glutathione reduced assay (Habig et al., 1974). Briefly, A mixture containing 2.0 M Pyrophosphate buffer(pH.7.4), 0.2 M of 5,5′-dithio-bis-[2-nitrobenzoic acid], and 50 µl of 10% tissue homogenate. The assay was based on the rate of formation of TNB-chromophore from the reaction of DTNB with intracellular GSH content. The TNB complex has a maximum absorbance of 412nm. The TNB complex formation rate was considered proportional to the GSH content of the tissue samples and the concentration of GSH was expressed as U/mg protein. The reaction mixture devoid of tissue homogenate served as blank.

3.14 Estimation of NO:

Griess reagent was utilized to assess the total nitrate/nitrite levels in the gastric mucosa, in order to determine the NO level (Li et al., 2017). Simply said, 50 mM potassium phosphate buffer (pH 7.8) was used to create the stomach homogenates.

After 10 minutes at 4 °C and 4000 rpm, the homogenates were centrifuged. The absorbance at 540 nm was measured after 10 minutes after adding 50µl of Griess reagent (0.1 % N-(1-naphthyl) ethylene diamide di hydrochloride, 1% sulfanilamide in 5% phosphoric acid) to 50 µL of supernatant. The standard curve for this assay was created using sodium nitrite as the standard; the concentration was determined by measuring the nitrite that was produced as a result of NO oxidation.

3.15 Immunohistochemical localization and evaluation of gastric cellular proteins; COXI, COXII, BAX, BCL2, HSP70, Caspase-3, PCNA & TNF-α

To prevent the stomach tissues from being excessively deformed they were dehydrated by immediately immersing them in ethanol solutions at increasing concentrations (70%, 90%, and 100%) for one hour each using the molten paraffin infiltration procedure. Fully dehydrated tissues were embedded in paraffin.
. The tissue block was cut into 5µm sections using an RM2125 RTS Leica rotary microtome.

. The tissue sections were placed on glass slides and kept for future use at room temperature. The tissue embedded slides were deparaffinized using xylene and then rehydrated using ethanol at progressively lower concentrations (100%, 90%, and 70%) for ten minutes at a time. Following a 10-minute PBS immersion, slides were blocked for 30 minutes at room temperature using goat serum 1:100 (Santa Cruz Biotechnology, Inc., CA, USA) in PBS inside a humidified chamber. The slides were incubated overnight at 4 °C in a humidified place using primary antibodies. After washing the slides in PBS, they were incubated for 3 hours at room temperature with secondary antibodies: HRP-conjugated goat-anti-rabbit (1:500, Mumbai, India) and HRP-conjugated goat-anti-mouse IgG (1:500, Santa Cruz Biotechnology Inc. Dallas, USA). After that, the slides were washed 3 times for 5 minutes each, and the slides were incubated for 5 minutes in a 0.05% diaminobenzidine (DAB) solution (3, 3'-diaminobenzidine in 5 nM Tris pH 7.6). Following 5 minutes of hematoxylin counterstaining, the slides were immersed in tap water, and the tissues were dried using ethanol at increasing concentrations (70, 90%, and 100%) for 10 minutes each.

After that, the slides were cleaned in xylene and mounted using DPX mountant. Using a Nikon binocular microscope (Model E200, Nikon, Tokyo, Japan), the slides were examined and photographed (Jeremy et al.,2019).

3.15.1 Details of antibodies used for immunohistochemistry

Antibody	Host	Dilution	Supplier	Catalog no
Primary Antibody				
Active caspase-3	Mouse	1:100	St.John's Lab	STJ97448
BAX	Rabbit	1:200	Santa cruz Biotechnology	SC6236
BCl2	Mouse	1:100	Santa cruz Biotechnology	SC7832
HSP70	Mouse	1:200	Santa cruz Biotechnology	sc-32239
C0X1	Rabbit	1:100	Elabsciences	E-AB-68405
COX2	Rabbit	1:200	Elabsciences	E-AB-17010
TNF-α	Mouse	1:100	Elabsciences	E-AB-33121
Secondary Antibody				
Rabbit IgG-HRP	Goat	1:400	Elabsciences	E-AB-1002
Mouse IgG-HRP	Goat	1:400	Elabsciences	E-AB-1001

3.16 Quantification of protein expression by Western blotting

Rats from each selected groups were sacrificed, and their stomachs were collected and homogenized for each group in lysis buffer (50 Mm Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 1 mM PMSF, and 1 mM EDTA). Further samples were then centrifuged for 10 minutes at 10,000 rpm in 4°C for western blot analysis (Annie et al., 2019). Bradford's technique was used to determine how much protein was present in the samples' supernatant (Bradford, 1976). SDS sample buffer (62·5 mM

Tris, 2% SDS, 10% glycerol, 1% mercaptoethanol, and 0·003% Bromophenol Blue, pH 6·8) was then used to denature the samples by boiling them for five minutes. 10% SDS-PAGE was loaded with 50 µg/well of each groups and a protein marker. The gel was then electrophoresed for two hours at 100 V. After that, the resolved proteins were put onto a polyvinylidineflouride membrane (Millipore India Pvt. Ltd., Bangalore, India) and left for twenty-five minutes using a Medox-Bio Mini Semi Dry Blotting MX-1295-01. Following a 30-minute incubation period at room temperature, the blots were blocked using 5% non-fat dried milk with PBS (10 mM, 7.5 pH) and 0.1% Tween20. Primary antibodies diluted in blocking solution were then incubated for the entire night at 4°C. The details of antibodies have been given in Table 3.16.1. The blots were washed with PBS-Tween20 for 2 changes and incubated with horse radish peroxidase-conjugated secondary antibody (1:4000) for 3 h at room temperature. After washing with PBS-Tween20, the blots were finally detected by chemiluminescence (ECL) (Cat # 1705060, BioRad, Hercules, CA, USA) and developed with x-ray film. The protein band was analyzed using ImageJ (1.38x, NIH, Bethesda, MD, USA). (Jeremy et al., 2019).

3.16.1 Details of antibodies used for western blot analysis:

Antibody	Host	Dilution	Supplier	Ctalog no.
Primary Antibodies				
COX 1	Rabbit	1:1000	Elabsciences	E-AB-68405
COX 2	Rabbit	1:2000	Elabsciences	E-AB-17010
BAX	Rabbit	1:1000	Santa cruz Biotechnology Inc	SC6236
BCl2	Mouse	1:1000	Santa cruz Biotechnology Inc	SC7832
PCNA	Rabbit	1:1000	Santa cruz Biotechnology Inc	Sc-7907
HSP70	Mouse	1:1000	Santa cruz Biotechnology Inc	sc-32239
Active Caspase-3	Mouse	1:1000	St,John's Lab	STJ97448
β -tubulin	Mouse	1:1000	Elabsciences	E-AB-20033

β-actin	Mouse	1:1000	Elabscience	E-AB-48018
Secondary Antibodies				
Rabbit IgG-HRP	Goat	1:4000	Elabsciences	E-AB-1002
Mouse IgG-HRP	Goat	1:4000	Elabsciences	E-AB-1001

3.17 Evaluation of gastric apoptotic cells by TUNEL assay (Terminal deoxynucleotidyl transferase dUTP nick end labelling)

The terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) assay kit was used to conduct the experiment in accordance with manufacturer protocol. Employing Apo-Bru-IHC following the manufacturer's instructions, the fixed-stored stomach tissues were prepared for the TUNEL analysis using an in-situ DNA fragmentation assay kit (Catalog #403-50. BioVision Inc. Milpitas, CA, USA).

In summary, the slides were deparaffinized, rehydrated, and then submerged in PBS before being treated with proteinase K (1:100 in 10 mM Tris pH 8) for 20 minutes at room temperature.

PBS was used to rinse the slides once again. By immersing the slides in a methanol+H_2O_2 solution (30% H2O2 1:10 in methanol) for five minutes, endogenous peroxidase was eliminated. i.e., Tthe sections had an incubation period of one hour at 37 °C with the labeling reaction mixture (5X reaction buffer) in 50 µl, terminal deoxyribonucleotidyl transferase (TdT) enzyme bufer 3.75 µl, bromodeoxyuridine triphosphate (Br-dUTP) solution 40 µl, and 161.25 µl double-distilled water". Slides were rinsed with PBS, blocked with blocking buffer for ten minutes, and then immediately incubated in a dark, humid room for approximately one and a half hours with an antibody solution (Anti-BrdU-Biotin antibody diluted with blocking buffer in a ratio of 1:19). Following another PBS washing, the slides were incubated for 30 minutes at room temperature with conjugate solution (200X conjugate combined

with blocking buffer in a 1:200 ratio). Following a PBS wash, the slides were incubated in a 3, 3′-diaminobenzidine (DAB) solution for duration of 15 minutes. Slides were counterstained for three minutes with methyl green solution after being washed with water. After dehydrating the slides for ten minutes at ethanol concentrations of 70%, 90%, and 100%, they were cleaned in xylene and mounted using mounting material. The apoptotic cells that were positive for TUNEL were seen as brown spots/cells.

3.18 Statistical analyses

All data were expressed as mean ± SEM. Normality distribution of the variables was tested using one sample Kolmogrov-Simirnov test. The results were analyzed by one -way analysis of variance ($p < 0.05$) followed by Tukey's test for post hoc comparisons by Graph pad prism.

CHAPTER 4

RESULTS

A. *Mallotus roxburghianus* Mull.Arg.

RESULTS:

4.1 Phytochemical analysis of methanol extract of *M. roxburghianus* (MRME)

LC-MS/MS chromatogram profile of MRME highlighted the presence of diverse phytocompounds as bergenin, betulinic acid, gallic acid, hesperidin, tannic acid, myrcetin, luteolin, kaempferol, quercetin, protocatechuic acid, hesperitin, gentisic acid, naringenin, ellagic acid, chlorogenic acid, rutin, genistein, chrysin and coumarin. Bergenin (35,321 mg/g), betulinic acid (13426.58 mg/g), gallic acid (6034.56 mg/g), hesperidin (4015.85 mg/g), tannic acid (3514.36 mg/g) and myrcetin (1288.88 mg/g) were found to be the most abundant phytocompounds in MRME. The other pharmacologically active compounds quantified were luteolin, kaempferol, quercetin, and naringenin to the tune of 752.35, 564.24, 545.61, 310.65 mg/g, respectively (Table 4.1). Protocatechuic acid, hesperitin, rutin, gentisic acid, chlorogenic acid, ellagic acid, coumarin, genistein and chrysin were also detected and present in lesser quantity.

Table 4.1 Identification and quantification of the most abundant phytocompounds present in methanol leaf extract of *M. roxburghianus* by LC-MS/MS.

Phytocompounds identified	**RT (min)**	**PI (m/z)[a]**	**MS2 (CE)[b]**
Tannic acid (Polyphenol)	0.94	182.80	125 (23), 79 (35)
Bergenin (Phenolic acid)	1.25	329.05	312 (26), 250 (13), 235 (18), 208 (54), 192 (42)
Betulinic acid (Terpenoid)	3.91	394.90	289 (29), 313 (48), 266 (30), 215, 155
Myricetin (Flavonoid)	7.18	318.15	177 (71), 152 (62), 138
Hesperidin (Flavonoid)	7.25	610.85	305 (44), 467 (19)
Gallic acid (Phenolic acid)	7.51	168.05	124 (12), 80 (27)

[a]PI: Parent ion (m/z) – molecular ions of the standard compounds (mass to charge ratio).

[b]MS2: multiple reaction monitoring fragments for the related molecular ions and CE refers to related collision energies of the fragment ions.

4.2 *in vivo* acute toxicity

The rats treated with MRME at 1000 or 3000 mg/kg doses throughout 15 days exhibited no signs of mortality, hepatic or renal toxicity symptoms and abnormal physiological and behavioural changes based on clinical and histopathological observations. Renal and liver function tests of rats after 14 days of MRME acute toxicity study showed a normal range of serum alanine aminotransferase (50.16–53.18 IU/L), aspartate aminotransferase (149.06–152.90 IU/L), urea (4.91–5.37 mmol/L) and creatinine (49.03–52.76 mmol/L) levels.

4.3 Gastric lesion evaluation, gastric pH, GWM content, ulcer index and ulcer preventive index

Absolute ethanol produced uneven and corroded GM folds, broad, deep and perceptible hemorrhagic lesions, deep ulcers and perforations in the GM of UC compared to C groups (Fig. 4 A and B). Conversely, the rats pretreated with OM (Fig. 4C) or MRME extracts at doses of 5–100 mg/kg (Fig. 4D–H) exhibited significantly lesser areas of spot ulcers, hemorrhagic streaks, thin ulcers and no perforations. Nevertheless, no sign of ulcer formation and presence of smooth and flat GM folds like the normal gastric tissues were observed at MR200 evidence of the protection of the GM (Fig. 4 I). GM damage and ulcer formation induced by absolute ethanol were significantly inhibited by MRME in a dose-dependent manner. Reduction of ulcer formation and protection of GM folds were witnessed in MRME pretreated rats but most prominent in MR200 treatment which was comparable to that observed in the OM treatment as well as in the C groups (Fig. 4). The administration of absolute ethanol showed a significant decrease in gastric pH (2.8) and increase in total acidity (18.2 mEq/L) in the UC group. On the contrary, rats pre-treated with MRME demonstrated a significant upsurge in the pH of the gastric contents (5.9–6.5) and a decrease in total acidity (9.2–12.8 mEq/L) compared to OM (pH, 6; total acidity, 10.2 mEq/L) and C (pH, 6.6; total acidity, 9.33 mEq/L) groups rats (Fig. 4.2A). The quantity of alcian blue binding in the GWM was significantly decreased in UC group (137.28 mg/g), while the rats pretreated with 5–200 mg/kg of MRME displayed a significant increase in alcian blue binding (196.26–490.75 mg/g)

in the GWM corroborate the protective action of MRME in inhibiting the GWM depletion (Fig. 4.2B). The absolute ethanol treated rats exemplified an ulcer score of 36 and an ulcer index of 3600. The pre-treatment of rats with MRME at doses of 5–100 mg/kg significantly reduced the ulcer formation in the GM (ulcer score, 13.2–28.34; ulcer index, 1320–2834) and increased the gastric tissue protection (ulcer preventive index, 21.28–63.33%) compared to the omeprazole (ulcer score, 20.8; ulcer index: 2080; ulcer preventive index, 42.22%). No ulcer formation (ulcer score, 0) and total comprehensive gastric tissue protection (ulcer preventive index, 100%) was accomplished in 200 mg/kg dose of MRME (Fig. 4C and 4D).

Fig 4.1 Gastro-protective effect of *M. roxburghianus* methanol leaf extract (MRME) on morphpogical appearance of the dissected stomachs' gastric mucosa in the Wistar albino rats; (A) Normal control group, (B) Ulcer control group pretreated with absolute ethanol (5 mL/kg) exhibited corroded gastric mucosal folds, broad, hemorrhagic lesions of ulcers (C) Omeprazole pretreated (20 mg/kg), (D, E, & F, G, H, I) rats pretreated with 5, 10, 20, 50, 100 and 200 mg/kg MRME respectively showing the signs of protection and restoration from ulcer formation in the gastric mucosa. Black arrows indicate the hemorrhage patterns of the gastric mucosa.

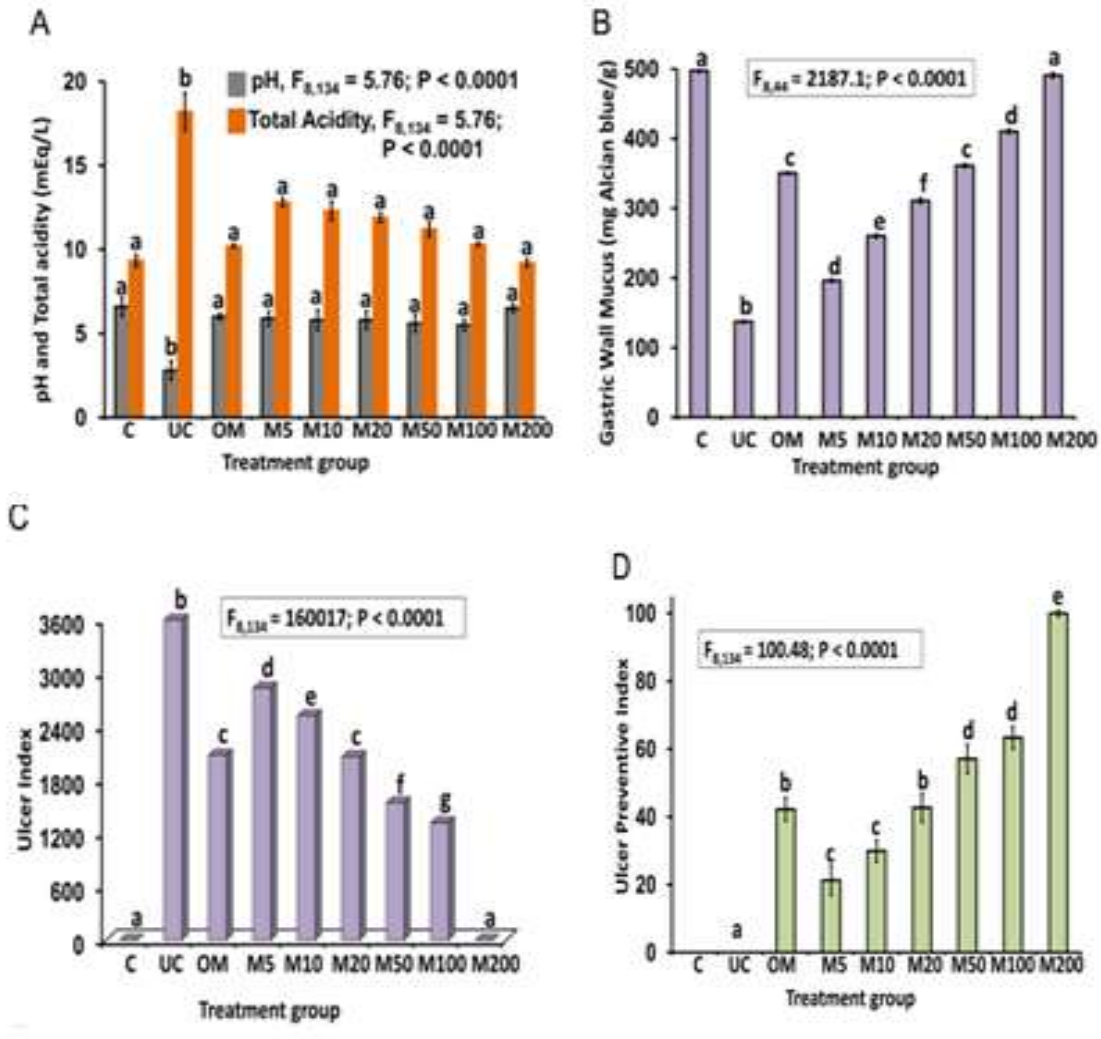


Fig. 4.2 Effects of *M. roxburghianus* methanol leaves extract (MRME) on: (A) pH and total acidity (mEq/L). (B) Gastric wall mucus content (alcian blue content/mg protein). (C) Ulcer index. (D) Ulcer preventive index. All values are expressed as the mean ± standard error of the mean. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test. Bar with different letter means statistical significant difference at $p < 0.05$ and similar letters are not significant. C, normal control group (0.5% carboxymethyl cellulose, CMC, 5 mL/kg); UC, ulcer control group (absolute ethanol, 5 mL/kg in 0.5% CMC); OM, omeprazole treated group (20 mg/kg in 0.5% CMC); M5, M10, M20, M100 and M200, MRME treated group at doses of 5, 10, 20, 100 and 200 mg/kg in 0.5% CMC, respectively.

4.4 Histopathology of GM lesions (H&E and PAS staining), eosiniphilic and mast cell count and Nitric oxide (MRME)

Histological examination (H&E staining) of the ulcer control group exhibited a severe gastric tissue injury and disruption of GM with necrotic lesions compared with normal control group (Fig. 4.4A and B). Severe interstitial hemorrhage, loss of the epithelial layer, distorted arrangement of glands, extensive edema, and infiltration of inflammatory cells were observed in mucosa and sub-mucosa (Fig. 4.4 B) of ulcer control group. Rats pretreated with 5, 10 or 20 mg/kg of MRME showed initiation of re-epithelialization of the ulcer fissure, a mild interstitial hemorrhage, and decrease in GM injuries, ulcer area formation, sub-mucosal edema and leukocyte infiltration in the sub-mucosal layer (Fig. 4.4 D–F). Nevertheless, pretreatment of 50, 100 or 200 mg/kg of MRME and omeprazole showed normal GM covered with surface mucus, more newly formed dilated gastric glands at the bottom and parietal cells with central rounded nuclei dispersed throughout the glands (Fig. 4.4 G–I). The intensity of PAS staining was increased significantly in the GM of MRME (Fig. 4.5 D–I) or omeprazole (Fig. 4.5 C) pretreated rats than ethanol treated ulcer control group (Fig. 4.5 B), signifying the protective effect of MRME against the mucin like glycoprotein content reduction induced by ethanol (Fig. 4.3 E).

The infiltration of eosinophils and mast cells attained a climax stage in the ulcer control group and then decreased gradually in the MRME and omeprazole treated groups (Fig. 4.3 E). Eosinophil counts in the GM and submucosa were both significantly higher in the ulcer control group (mucosa: 56.55 cells/mm^2; submucosa: 48.15 cells/mm^2) whereas the density of eosinophils in the MRME (mucosa: 20.16–38.15 cells/mm^2; submucosa: 20.68–45.05 cells/mm^2) or omeprazole (mucosa: 28.25 cells/mm^2; submucosa: 26.75 cells/mm^2) pretreated rats was decreased significantly (Fig. 4.3 F and G). The number of submucosal mast cells was significantly increased in the ulcer control group 59.16 cells/mm^2) than normal control group (22.42 cells/mm^2). Following the pretreatment with MRME or omeprazole, the mast cell count decreased to 25.18–49.05 cells/mm^2, respectively (Fig. 4.3 H). Further, significant decline in the number of intact and active/degranulated mast cells was noticed in the submucosal layer of MRME (active: 15.65–38.45 cells/mm^2; Intact:

6.56–12.52 cells/mm^2) or omeprazole (active: 20.66 cells/mm2; Intact: 10.15 cells/mm2) pretreated groups than ulcer control group (active: 42.56 cells/mm^2; Intact:15.65/mm^2) indicating the cytoprotective action of MRME through inflammatory mediators (Fig. 4.3H).

In the current study the group with ethanol induced ulcers demonstrated a noteworthy decrease in stomach nitric oxide (NO) levels when compared to the control group ($p<0.001$). While MRME pretreated and OM groups showed significant ($p<0.001$) restoration NO levels with highest NO level recorded at 200 mg/kg MRME dose suggesting the herb's anti-ulcer effectiveness (Fig. 4.6).

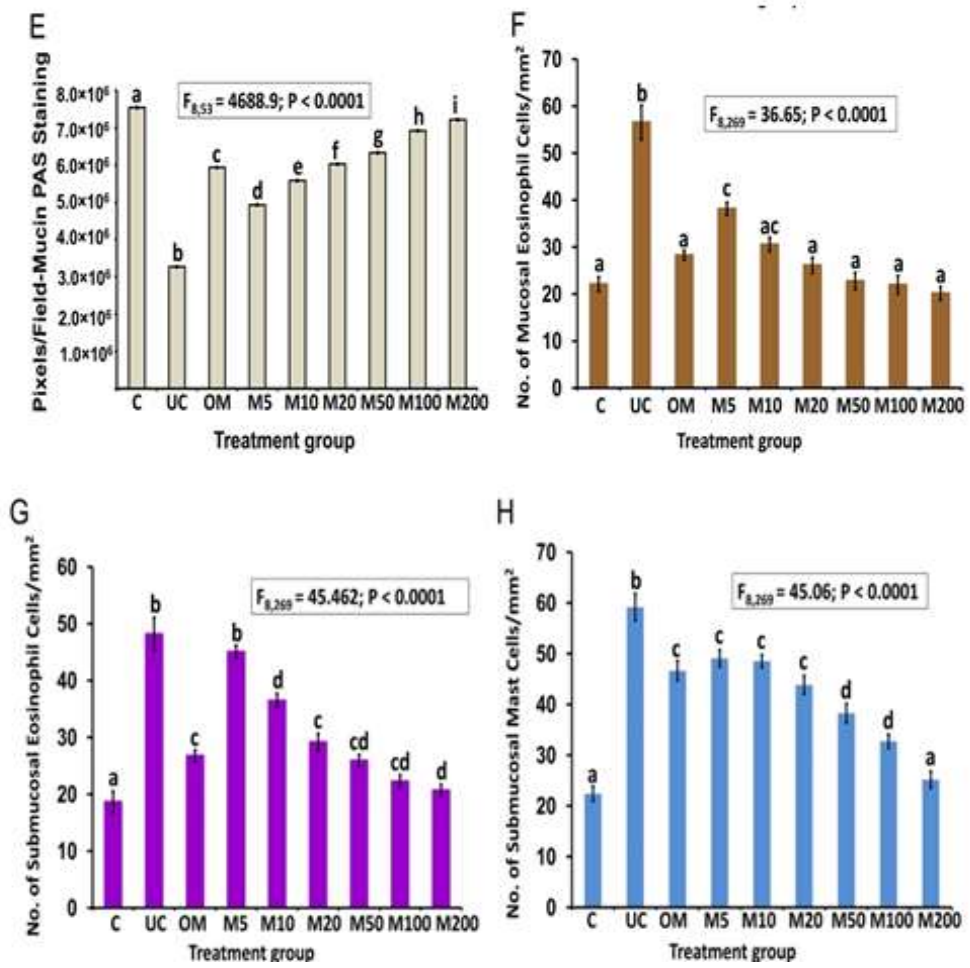


Fig. 4.3 Effects of *M. roxburghianus* methanol leaves extract (MRME) on: (E) Intensity of carmine PAS histochemical staining (pixels 106/field of the gastric tissues and their quantification with imageJ software. (F and G) Number of mucosal and submucosal eosinophils infiltration/mm^2/treatment group, respectively. (H) Number of submucosal mast cells/infiltration/mm^2. All values are expressed as the mean ± standard error of the mean. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test. Bar with different letter means statistical significant difference at $p < 0.05$ and similar letters are not significant. C, normal control group (0.5% carboxymethyl cellulose, CMC, 5 mL/kg); UC, ulcer control group (absolute ethanol, 5 mL/kg in 0.5% CMC); OM, omeprazole treated group (20 mg/kg in 0.5% CMC); M5, M10, M20, M100 and M200, MRME treated group at doses of 5, 10, 20, 100 and 200 mg/kg in 0.5% CMC, respectively.

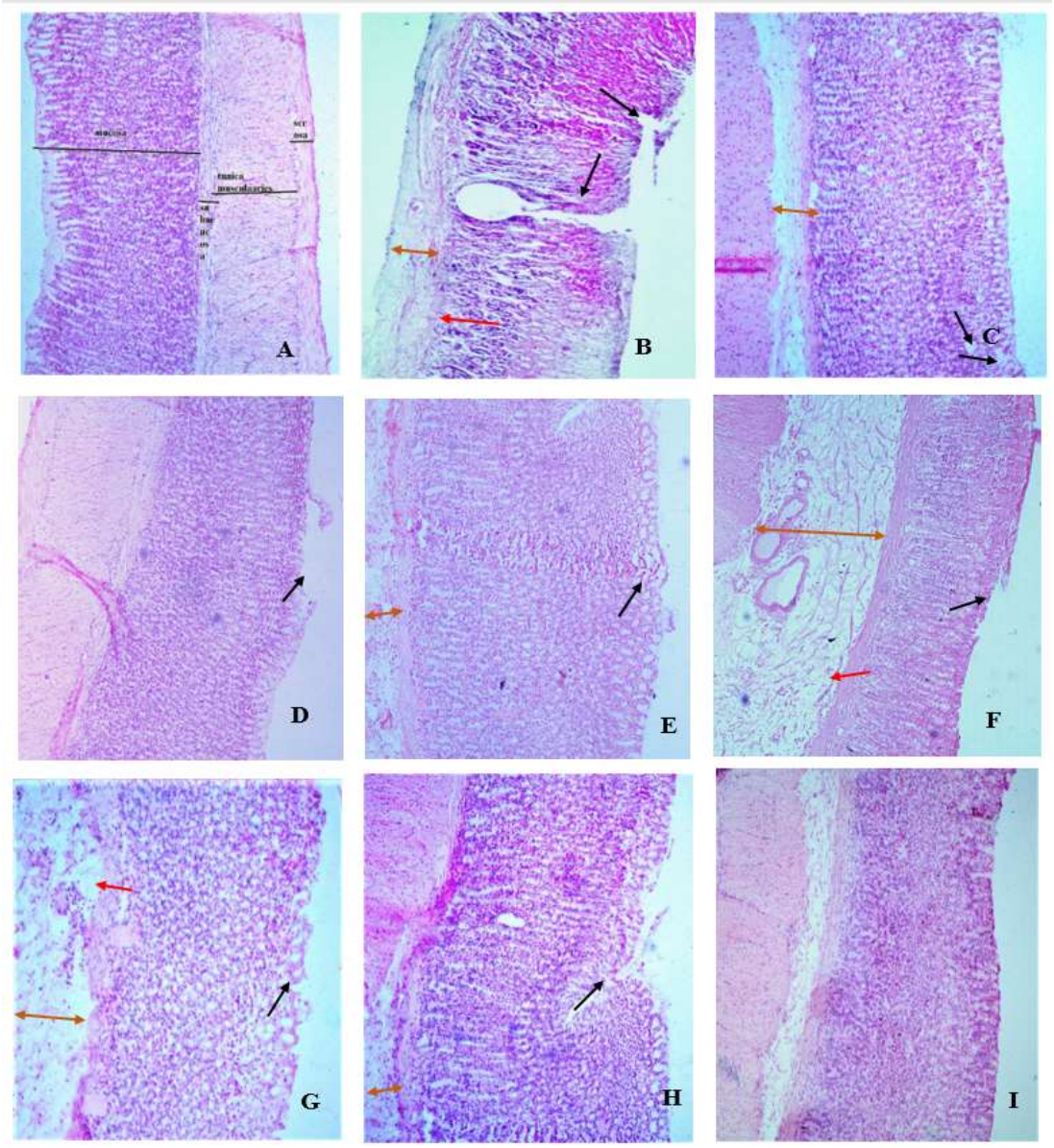


Figure 4.4(I) Histology of gastric mucosal (4X). In the normal control group (A), no disruption to the surface epithelium was observed. The ulcer control group (B) has severe disruption in the surface epithelium (black arrows) and necrotic lesions penetrate deeply into mucosa and extensive edema of submucosal layer (orange arrows) and leucocyte infiltration (red arrows) are present. The reference control group (omeprazole, 20 mg/kg) (C), mild disruption of the surface epithelium mucosa was recorded but deep mucosal damage is absent. Reduction of submucosal edema and leucocytes infiltration is seen. Rats received, 5mg/kg,10 mg/kg ,20mg/kg ,50mg/kg & 100mg/kg of the MRME (D-H) has mild to moderate disruption of surface epithelium with submucosal edema and leucocytes infiltration of submucosal

layer. Rats received 200 mg/kg of the MRME (I) has no disruption to the surface epithelium.

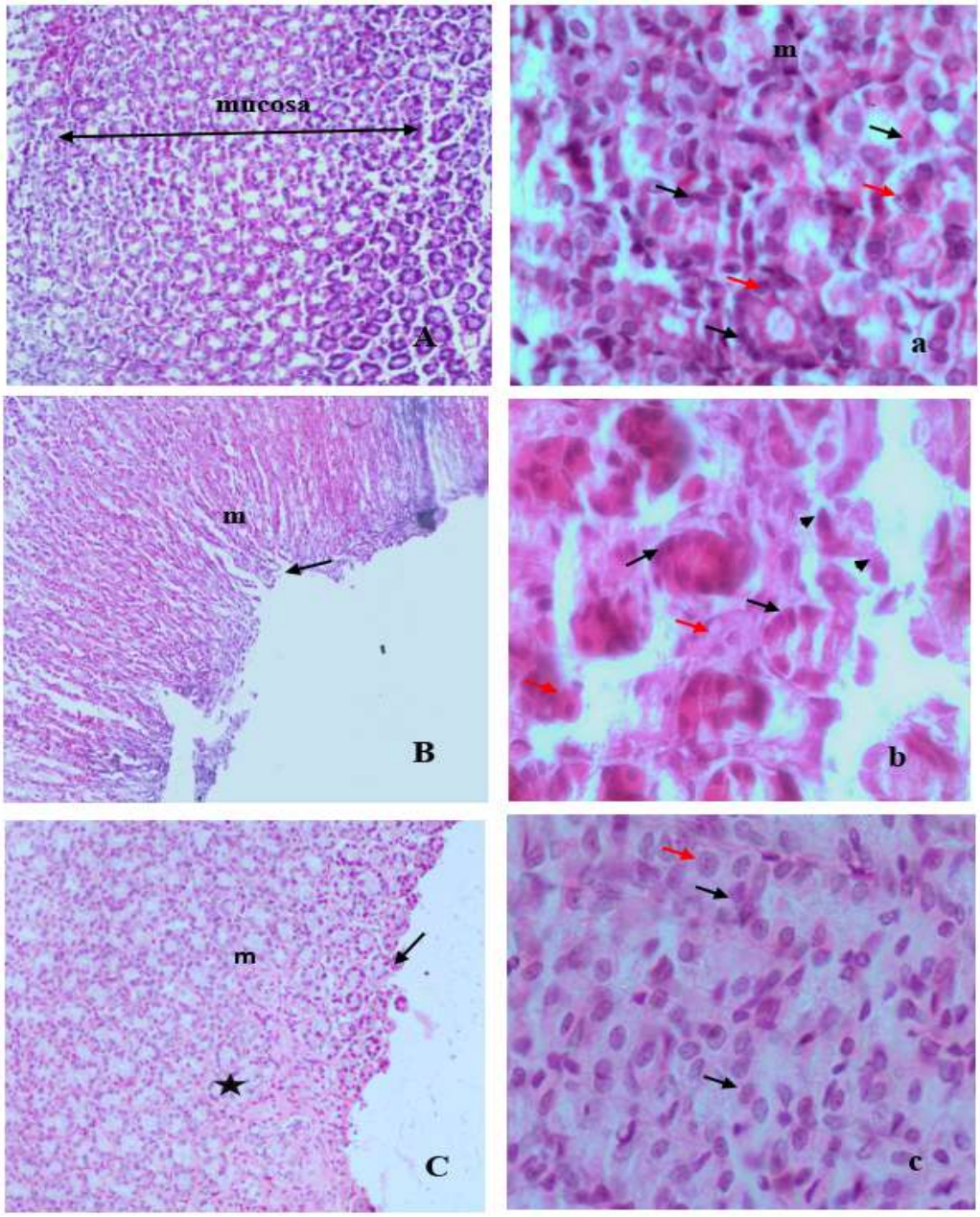


Figure 4.4(II) The histology of stomach of control group showing normal architecture of stomach mucosa(A); (a) normal structure of gastric mucosa showed multiple chief cells (black arrows) lining the base region of glands and many parietal cells (red arrow). Photomicrographs of the gastric mucosa from the gastric ulcer group showing: (B) gastric ulcer with desquamated cells (black arrow);(b) desquamated cells & pyknotic surface epithelial cells (arrow

head) (c) vacuolated parietal (red arrow) and Some deeply stained chief cells with dark pyknotic nuclei (black arrows) are also observed .photomicrograph of the mucosa from omeprazole group showed mild ulceration in the upper part, widening of gastric glands (★), damage of some cells (arrow) and many parietal cells appeared normal (red arrow)(A,B,C show 10X & a,b,c show 40X magnification).

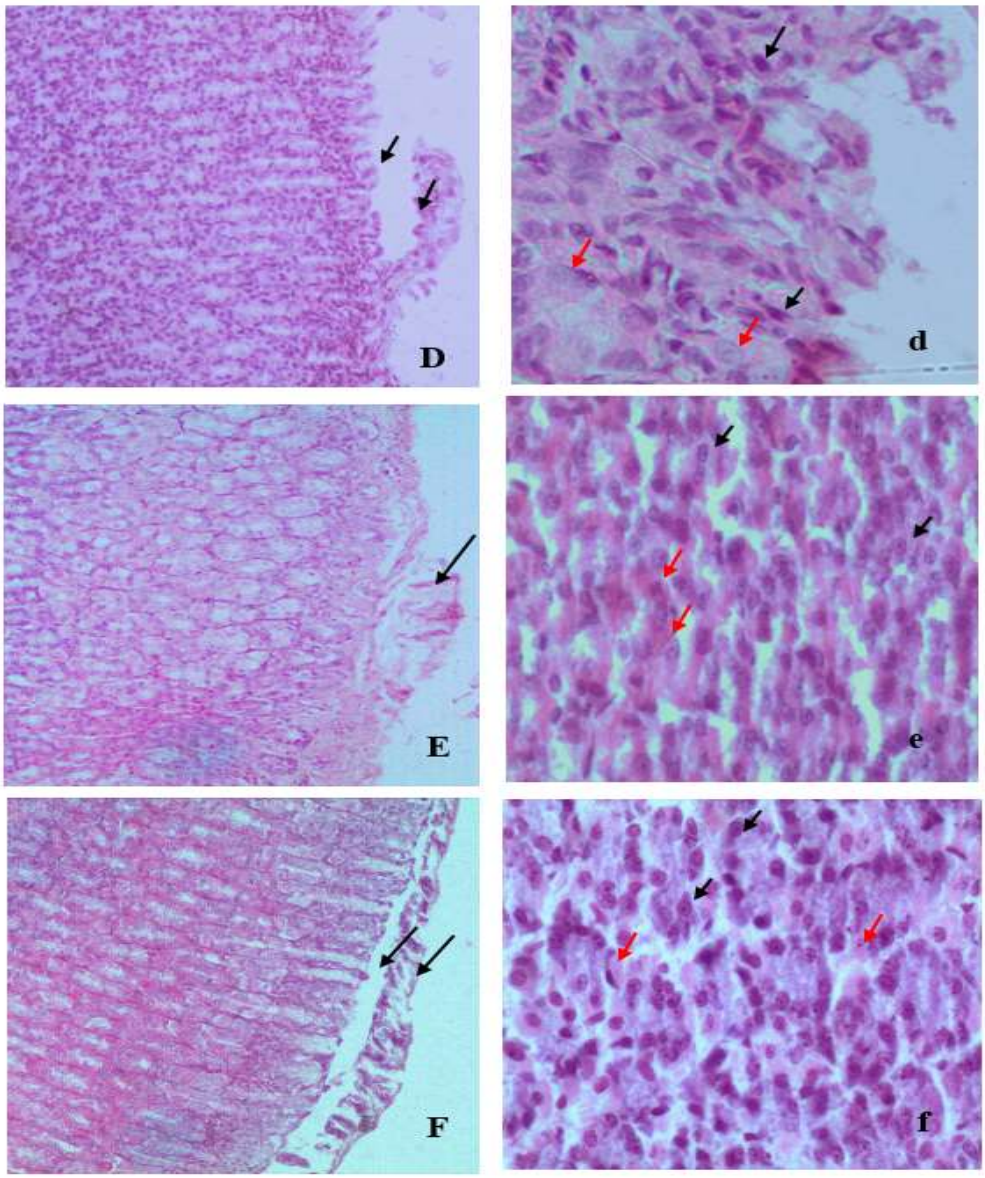
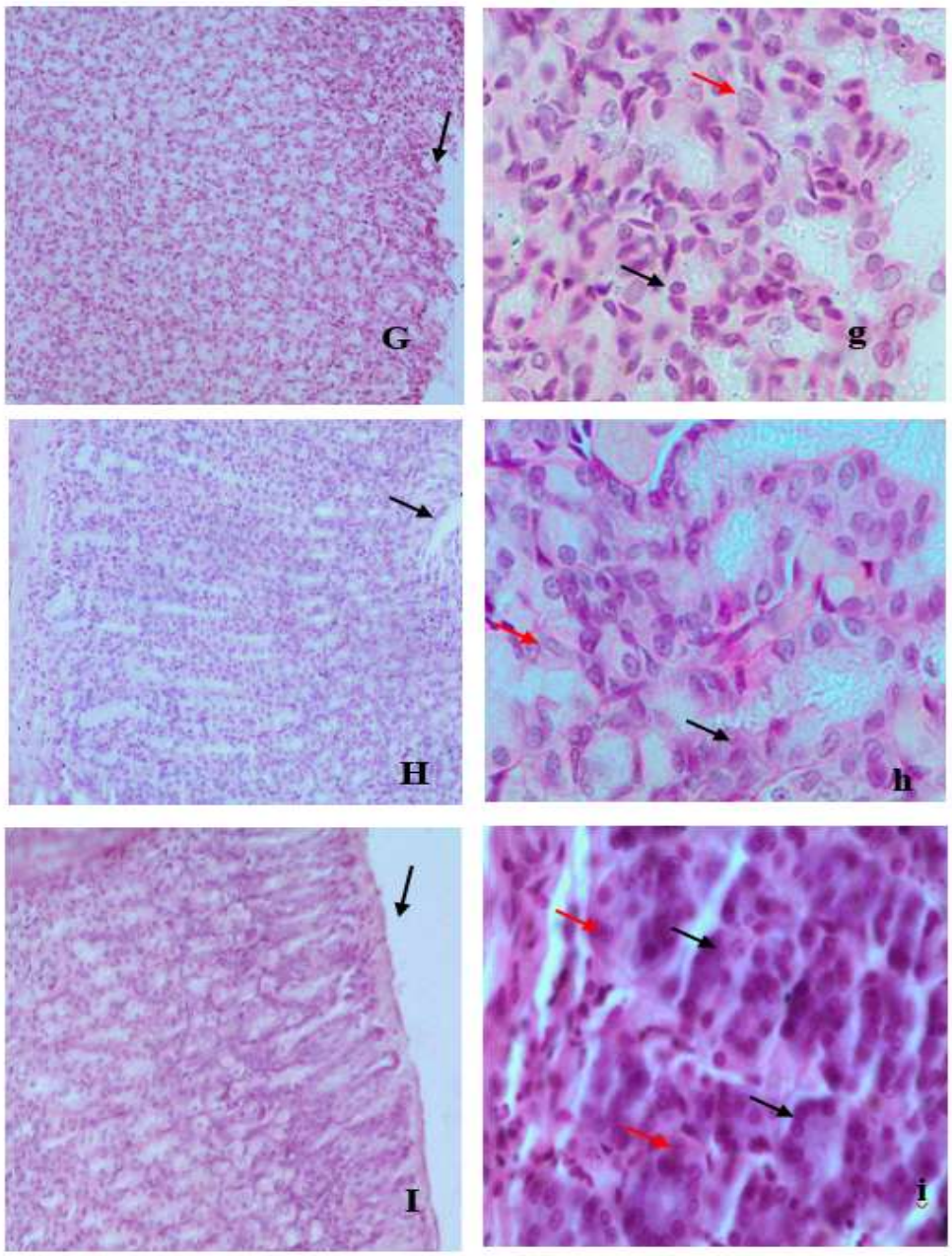


Figure 4.4(III) The histology of stomach pretreated with MRME (5,10 & 20ml/kg) group (D, E & F,10X magnification) exhibited distorted architecture of upper stomach part & exhibits severe surface mucous cell loss with desquamation of the lining epithelium into the lumen (black

arrow), few degenerated parietal cells (red arrow) and many dark chief cells with pycknotic nuclei (black arrow) (d, e & f,40X magnification).



igure 4.4(IV) The histology of stomach pretreated with MRME (50-100mg/kg; G & H,10X) exhibited ulceration of upper part, exhibits severe surface mucous cell loss with desquamation of the lining epithelium into the lumen (black arrow), fig.(g&h,40X) revealed disorganization & degenerated parietal cells (red arrow) and many dark chief cells with pycknotic nuclei (black arrow). The stomach of group of MRME(200mg/kg) showing (I,10X)

a near-normal gastric mucosa, showed improvement/restoration of stomach architecture in upper part as covered with mucous secretion (arrow) of superficial layer of the gastric mucosa; normal structure of most base layer cells; chief cells (black arrow) and parietal cells (red arrows) (fig. i:40X).

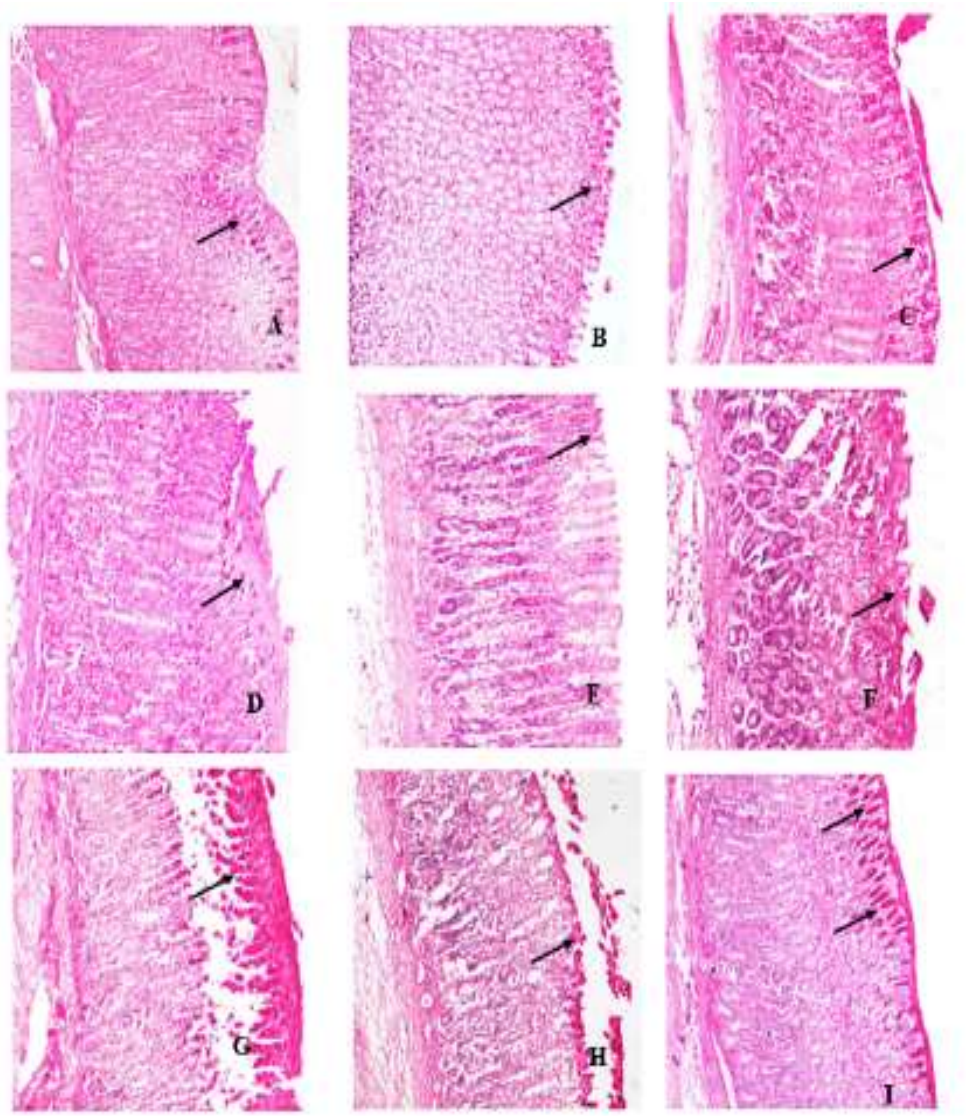


Figure 4.5 Effect of MRME on Periodic Acid Schiff (PAS)(10X) staining of stomach glycoproteins excretion on ethanol-induced gastric damage in rat. (A). The normal control group exhibited normal magenta color of the stomach mucosa (black arrow). (B). The ulcer control group exhibits a reduction of PAS staining of the stomach epithelial tissues with widespread mucosal injuries. (C). The omeprazole group exhibited

increased PAS staining. (D), (E), (F), (G), & (H) The experimental group exhibited low intensity of PAS stain & mucosal injuries, (I), the experimental group(200mg/kg) showed high intense of PAS stain (marked with black arrow).

4.5 Gastric oxidative stress marker (MDA) and cytoprotective antioxidant enzymes

The normal untreated control group showed the MDA, SOD and GST levels of 2.68 nmol/mg protein, 1.85 U/mg proteins and 9.27 U/mg protein, respectively in the gastric homogenate. A significant increase in MDA activity (3.66 nmol/mg protein) and a significant depletion of SOD (0.95 U/mg protein) and GST (3.27 U/mg protein) activities were noticed in the gastric homogenate of ethanol treated ulcer control group rats. In contrast, MRME and omeprazole pretreated rat groups demonstrated significantly lower MDA levels (MRME: 1.01–2.74 nmol/mg protein; omeprazole: 2.81 nmol/mg protein, Fig. 4.7J) as well as augmented SOD (MRME: 1.59–1.82 U/ mg protein; omeprazole: 1.62 U/mg protein, Fig. 4.7K) and GST (MRME: 3.82–9.12 U/mg protein; omeprazole: 4.02 U/mg protein, Fig. 4.7L) contents in the gastric homogenate.

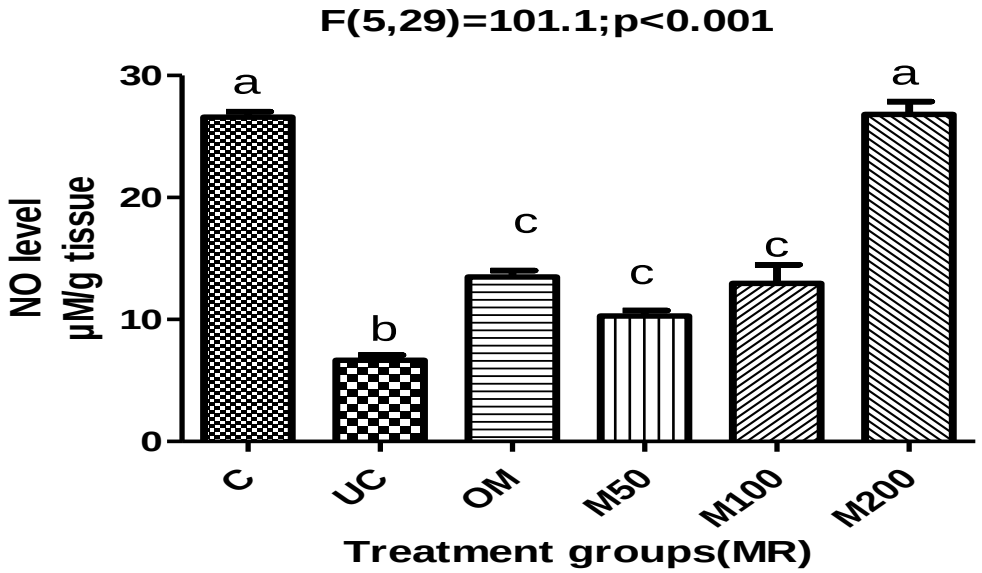


Fig. 4.6 Effects of *M. roxburghianus* methanol leaves extract (MRME) on Nitric oxide regulation. (A) Normal control group, (B)Ulcer group (C) Omez:(D);

(E);(F) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant.

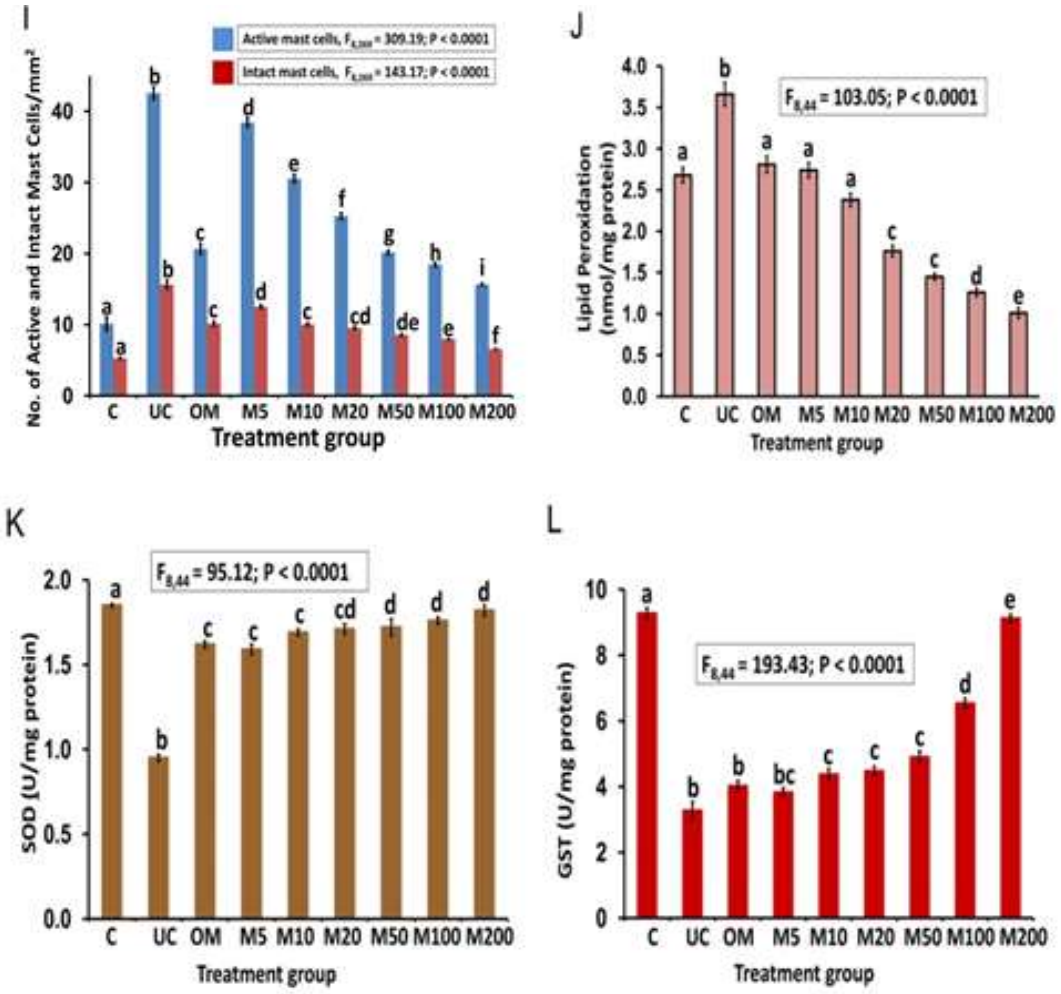


Fig. 4.7 Effects of *M. roxburghianus* methanol leaves extract (MRME) on: (I) Number of active and intact sub mucosal mast cells infiltration/mm^2 respectively (J) Lipid peroxidation (nmol MDA/mg protein). (K and L) Superoxide dismutase (SOD) and glutathioneS-transferase (GST) antioxidant enzymes scavenging activities (U/mg protein), respectively. All values are expressed as the mean ± standard error of the mean. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test. Bar with different letter means statistical significant difference at $p < 0.05$ and similar letters are not significant. C, normal

control group (0.5% carboxymethyl cellulose, CMC, 5 mL/kg); UC, ulcer control group (absolute ethanol, 5 mL/kg in 0.5% CMC); OM, omeprazole treated group (20 mg/kg in 0.5% CMC); M5, M10, M20, M100 and M200, MRME treated group at doses of 5, 10, 20, 100 and 200 mg/kg in 0.5% CMC, respectively.

4.6 Immunohistochemistry and western blot analysis of expressed proteins in gastric ulcer

4.6.1 Modulation of Cyclooxygenase (COX I and COX II) expression

COX I and COX II expressions in gastric mucosa of the control(C), ethanol induced ulcerated (U), Omeprazole pretreated (OM; 20mg/kg), MRME pretreated with M50 (50mg/kg), M100 (100mg/kg), and M200 (200mg/kg) in Fig 4.8(I& II) and 4.9(I & II)**.** Immunohistochemical staining in gastric mucosa showed a constitutive high expression of COX-I in normal control groups of rats. Ethanol induced ulcerated rat groups significantly lowered the COX- I expression compared with normal control group. Omeprazole pretreated groups COX-I expression was significantly reduced compared control. MRME pretreated groups showed significantly lowered expression in M50 and exhibited increased expression trend in M100 and M200, thus higher doses of MRME helped in the restoration of COX-I expression in the gastric tissues (Fig. 4. 8-II).

While immunohistochemical staining of gastric mucosa sections showed a low level of expression of COX-II in the normal control groups of rats(C). There is a significant inducible high-level expression of COX-II expression in the ethanol induced ulcerated rat groups. In the Omeprazole pretreated groups lowered the COX-II expression. In the MRME pretreated rat groups significant exhibited high level of expression in the M50 and M100 compared the normal control while in the high dose M200 showed low level of expression similar to that of normal control groups of COX II respectively (Fig. 4.9-II).

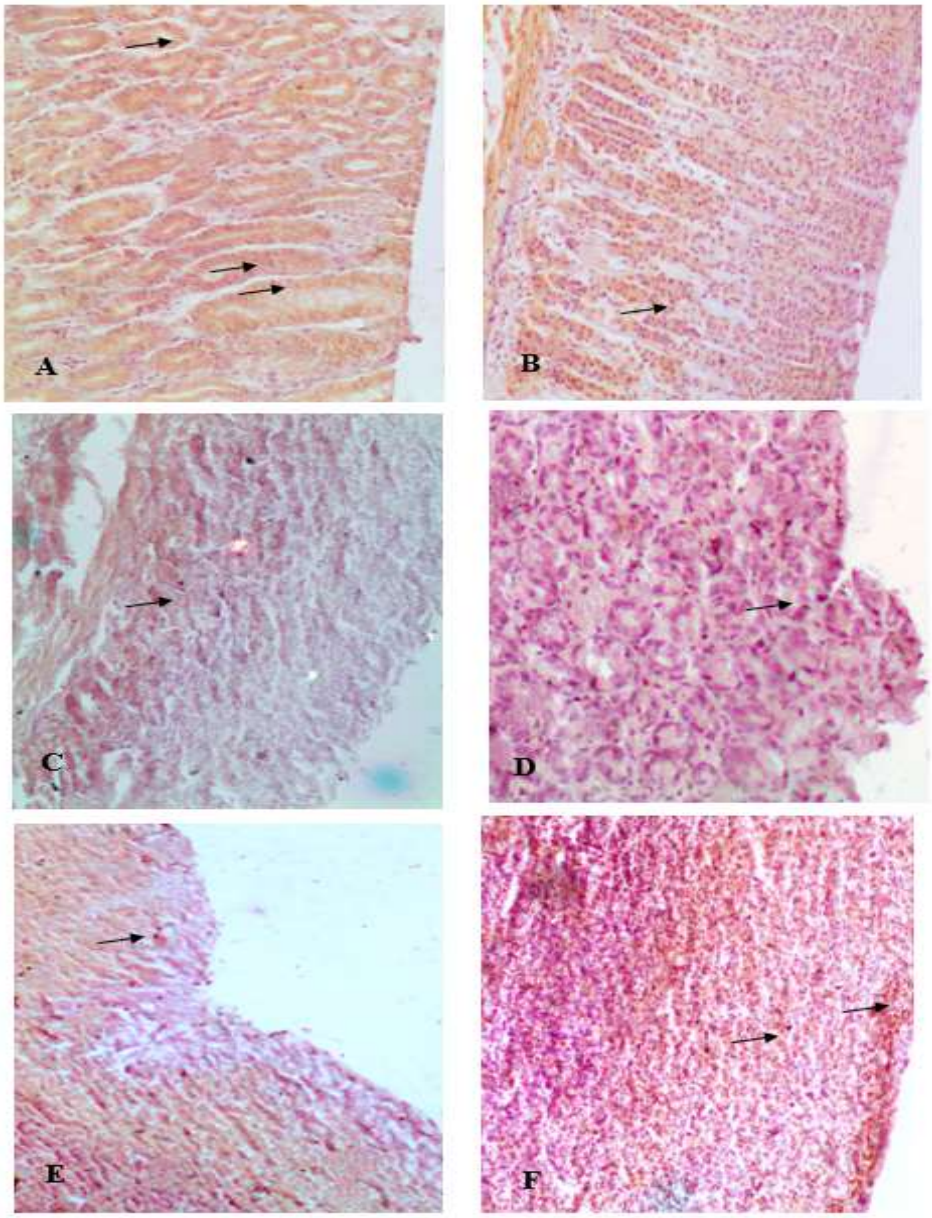


Figure 4.8(I). Representative photomicrographs showing COX-1 immunohistochemical staining (magnification 10X. (A)Normal control group, (B)Ulcer group (C) Omez:(D); (E); (F) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Immunopositive areas are brown in colour in which, black arrows indicate immunopositive cells.

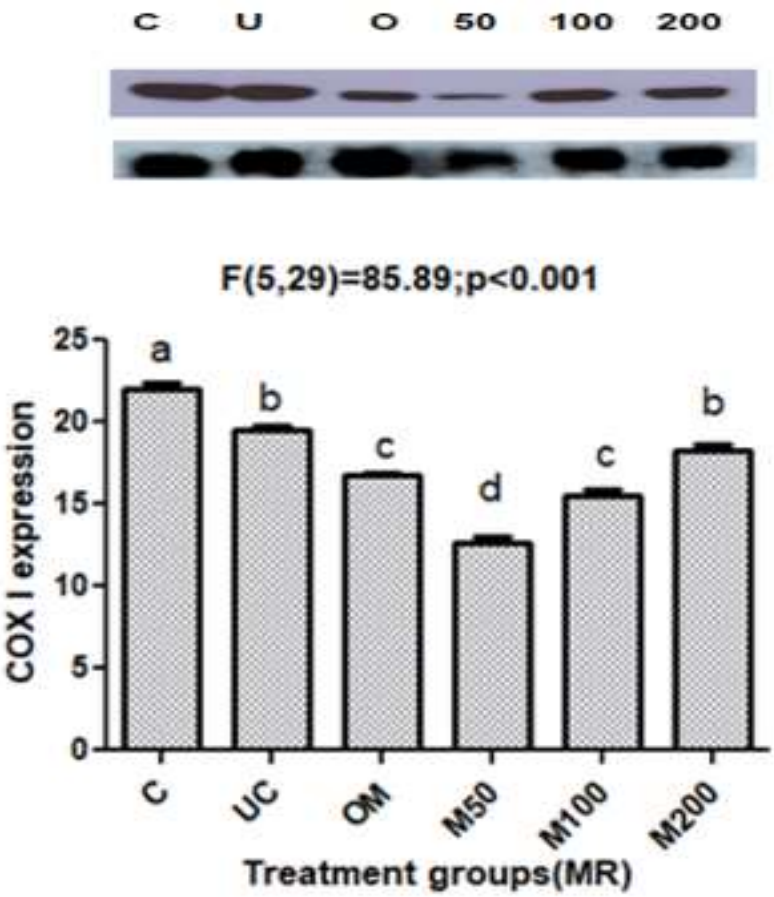


Figure 4.8(II) COX-I expression: The graph represents semi-quantification of COX-1 with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (M50); (M100); (M200) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Representative western blot image is also presented herewith. Statistical comparison was performed using one-way ANOVA followed by Tukey 's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.**

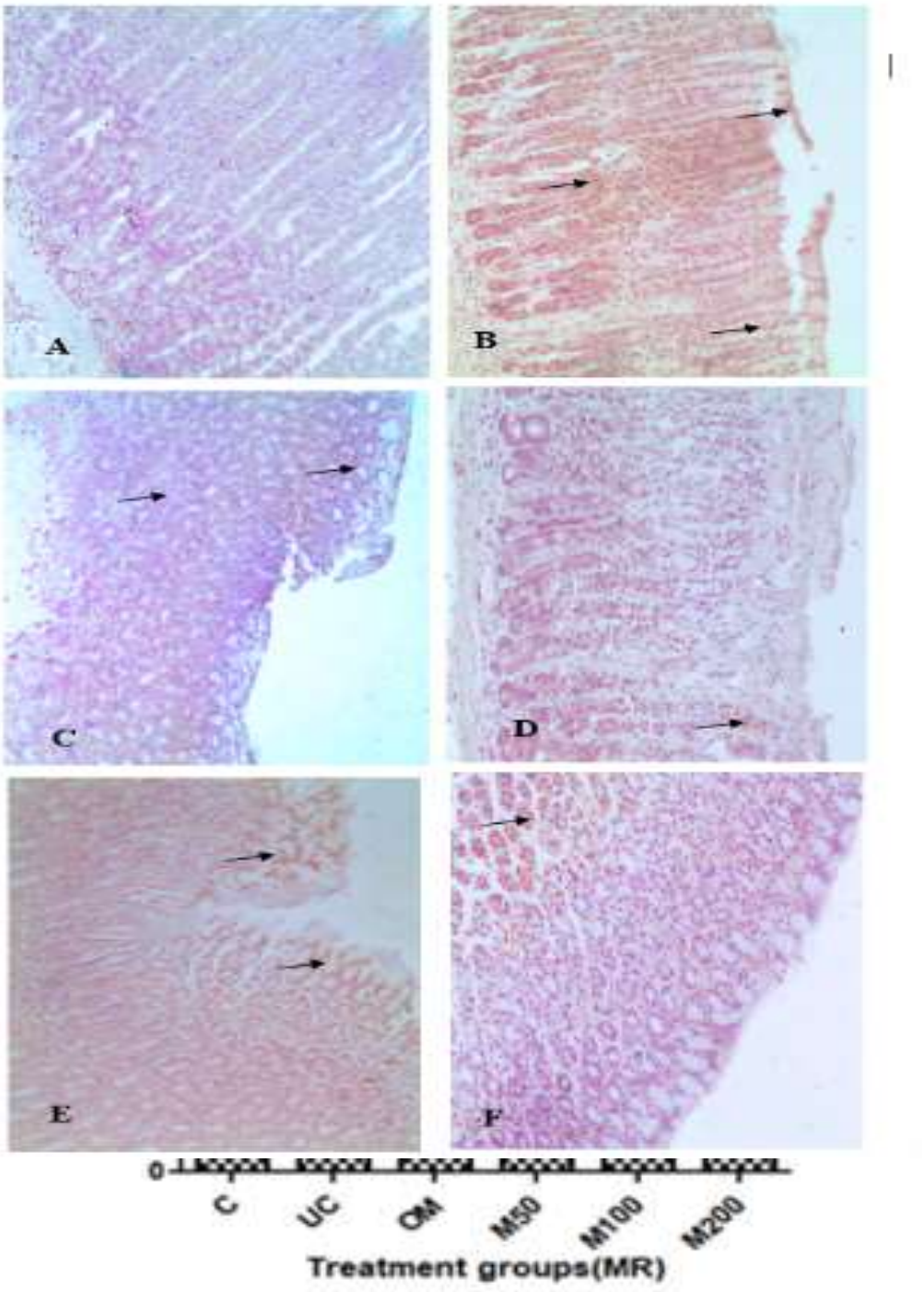


Figure 4.9 COX-II expression: The graph represents semi-quantification of COX-II with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (M50) ;(M100) ;(M200) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Representative western blot image is also presented herewith. Statistical comparison was

performed using one-way ANOVA followed by Tukey ‘s multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.**

4.6.2 BCl2 expression

The imunohistochemical results showed high level of expression of the anti-apoptotic BCL2 proteins in the normal control groups of rat. The BCL2 proteins expression was significantly reduced/lowered in the ulcer control groups compared normal groups, indicated the clear role apoptotic process in the gastric tissues. Rat pretreated with Omeprazole and MRME pretreated doses M50 and M100 again showed down regulated BCL2 protein expression in the gastric tissue. While M200 significantly enhanced/restored the level of expression of the BCL2 similar that of normal control group. Thus higher dose of MRME (M200) has noticeable protective and anti-apoptotic mode of actions against ethanol induction in the gastric tissue of rats. (Fig. 4.10 I & II).

4.6.3 BAX expression

The imunohistochemical results showed low/reduced level of expression of the pro-apoptotic BAX proteins in the normal control groups of rat. The BAX proteins expression was significantly higher in the ulcer control groups compared normal groups, indicated the clear role apoptotic process in the gastric tissues. Rat pretreated with Omeprazole and MRME doses M100 and M200 significantly ($p<0.001$) down regulated BAX protein accumulation in the gastric tissue denoting their noticeable protective and anti-apoptotic mode of actions against ethanol induction. (Fig. 4.11 I & II).

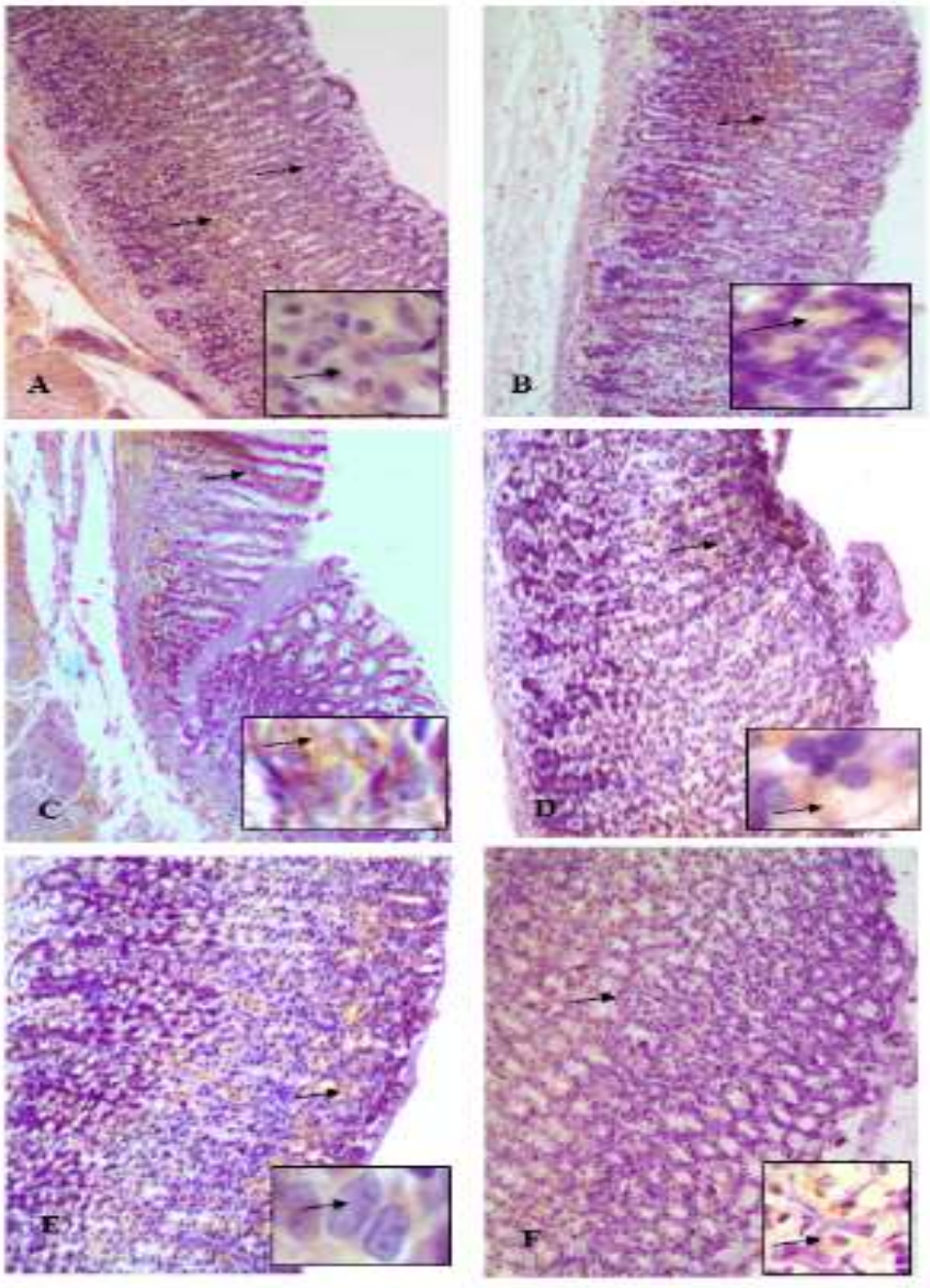


Fig. 4.10(I) BCL-2 expression: Representative photomicrographs showing BCL-2 immunohistochemically staining magnification 10X and in each image small box (40X). (A)Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Immunopositive areas are brown in colour showed by black arrows indicate immunopositive cells.

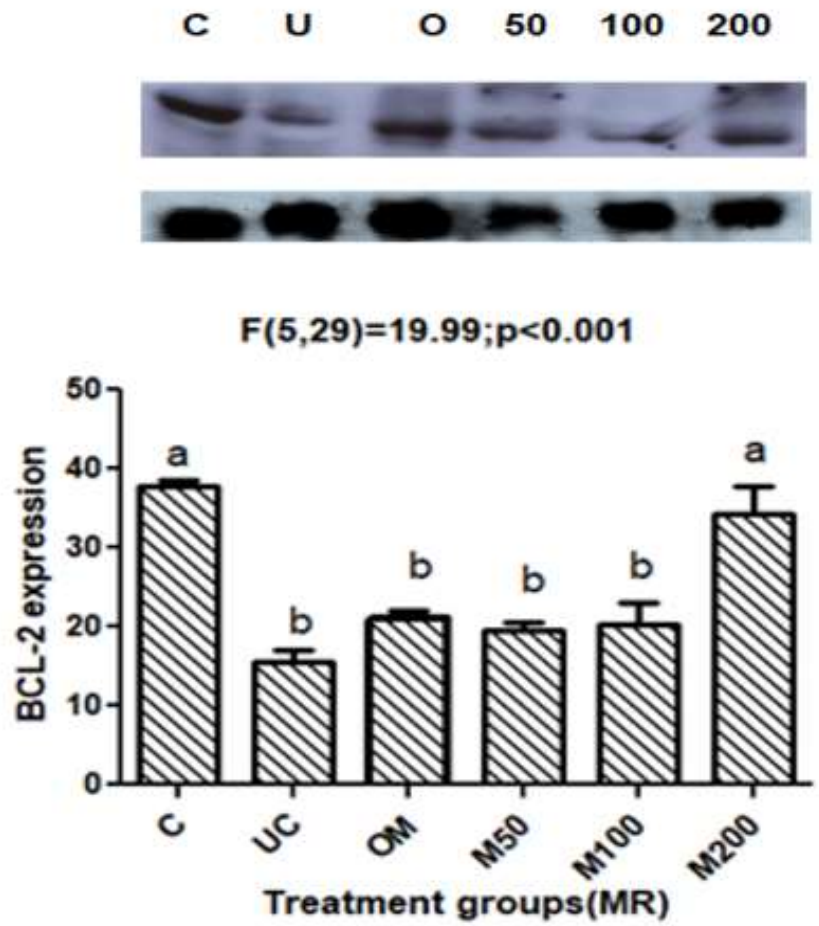


Figure 4.10(II) BCL-2 expression: The graph represents semi-quantification of BCL-2 with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (M50) ;(M100) ;(M200) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Representative western blot image is also presented herewith. Statistical comparison was performed using one-way ANOVA followed by Tukey 's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.**

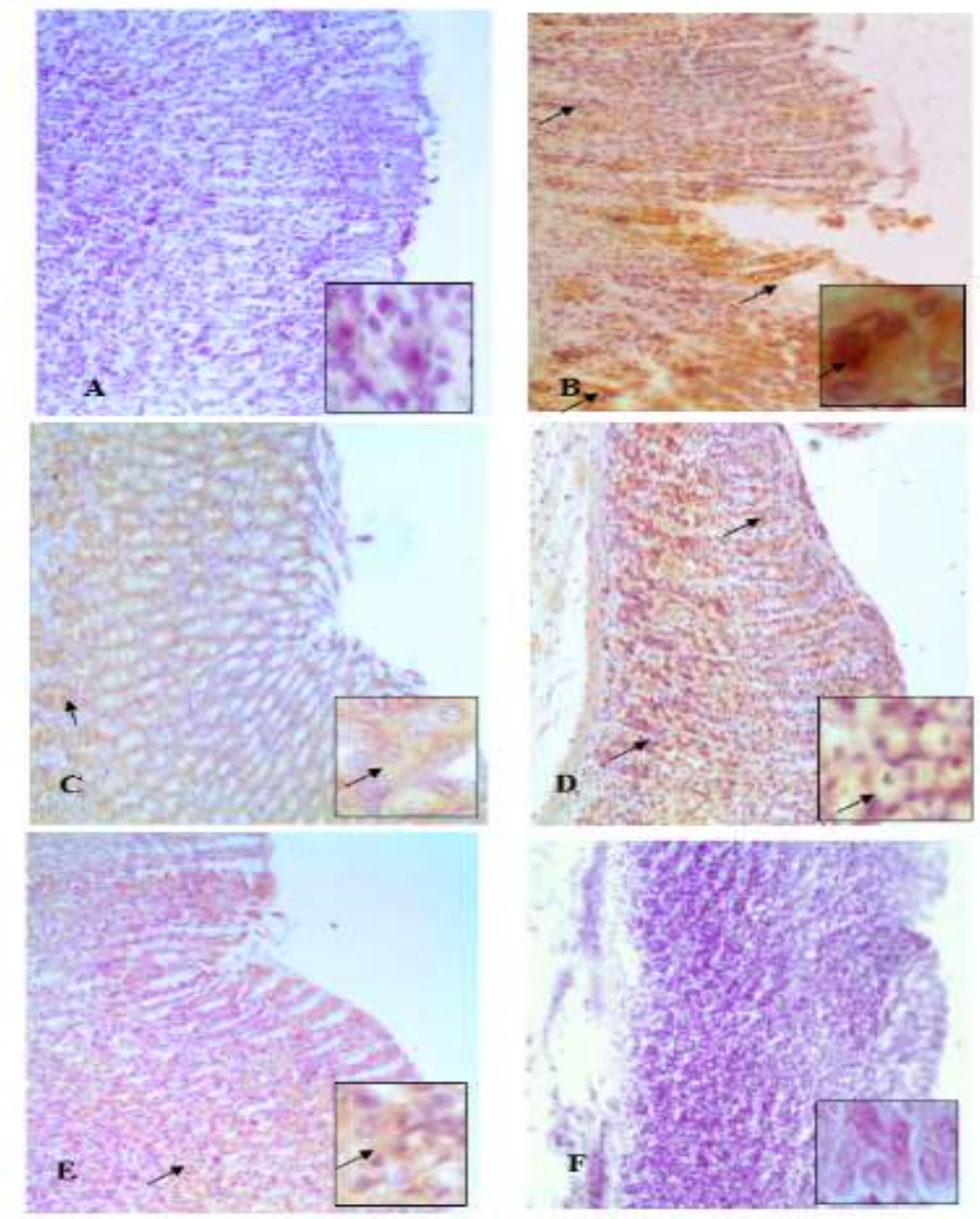


Fig. 4.11(I) BAX expression: Representative photomicrographs showing BAX immunohistochemically staining magnification 10X and in each image small box (40X). (A)Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Immunopositive areas are brown in colour showed by black arrows indicate immunopositive cells.

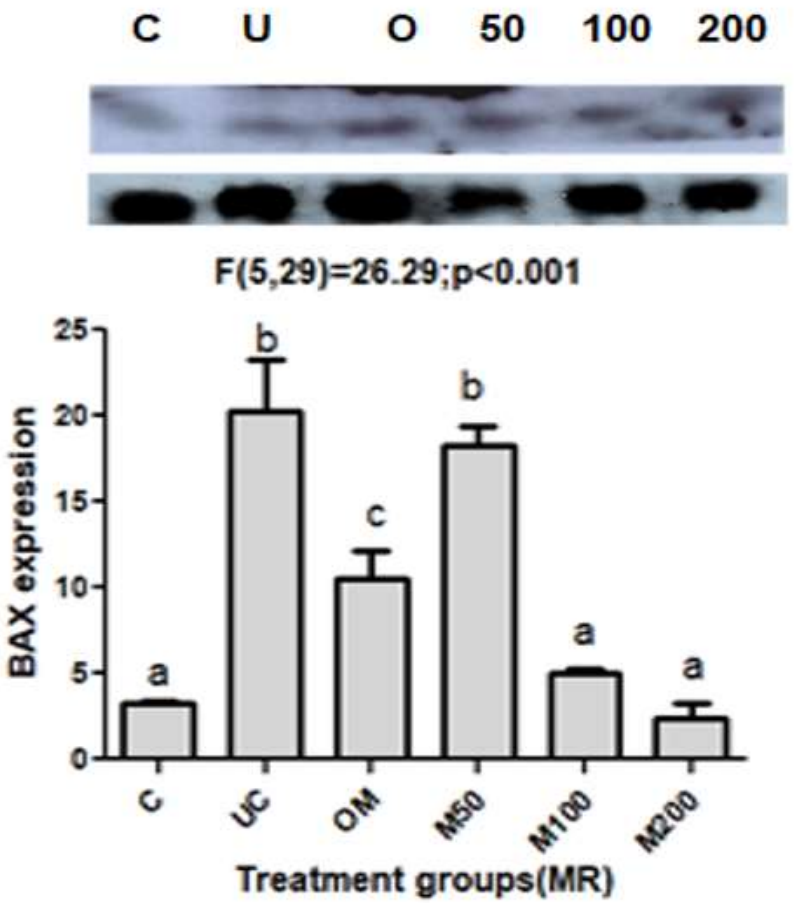


Fig. 4.11(II) BAX expression; The graph represents semi-quantification of BAX with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (M50) ;(M100) ;(M200) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Representative western blot image is also presented herewith. Statistical comparison was performed using one-way ANOVA followed by Tukey 's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.**

4.6.4 Caspase-3 expression

Immunohistochemical localization investigation revealed a strong reactivity/expression of Caspase-3 in the gastric mucosa of ethanol induced ulcerated groups evidence dark brown color statistically significantly ($p<0.001$) compared to normal control group. The rat pretreated with Omeprazole and MRME at doses M50, M100 and M200 significantly ($p<0.001$) down regulated Caspase-3 protein expression as evidence low reactivity in the gastric tissue. Thus, there is a noticeable protective and anti-apoptotic mode of actions against ethanol induction by MRME (Fig. 4.12 I & II).

4.6.5 HSP70 Expression

The imunohistochemical results showed significantly low/reduced level of expression of the heat shock protein (HSP70) in the normal control groups and ulcer control group of rat. The HSP70 protein expression was significantly up regulated in rats pre-treated with MRME at doses M50, M100 and M200 or omeprazole (brown color illustrates over-expression of HSP70 protein) while HSP70 expression is down regulated (less brown color expression) in ulcer control group($p<0.001$). Up regulated HSP70 protein expression in the gastric tissues pre-treated with MRME denoted their cell protecting ability against ethanol induced ulceration (Fig.4.13, I & II).

4.6.6 TNF-α expression (only Immunohistochemistry)

The imunohistochemical results showed significantly low/reduced level of expression of the pro-inflammatory cytokine TNF-α protein in the normal control groups of rat. The cytokine TNF-α expression was higher in the ethanol induced ulcerated control groups compared normal groups, indicating the role inflammatory process in the gastric tissues. Rat pretreated with Omeprazole and MRME doses M100 and M200 ($p<0.001$) down regulated TNF-α statistically significant in the gastric tissue denoting their anti-inflammatory mode of actions against ethanol induction (Fig.4.14 I & II).

4.6.7 PCNA expression (Only western blot)

The western blot results showed significantly very low/reduced level of expression of the proliferating cell nuclear antigen (PCNA protein) in the ethanol induced ulcerated control groups compared normal group indicated retardation/arrest of cycle in the gastric tissue. Omeprazole and MRME pretreated groups showed improved level expression in M50 and exhibited increased expression trend in M200, thus higher dose M200 of MRME helped in the restoration cell cycle regulation in the gastric tissues (4.15 I & II).

4.6.8 Tunnel assay for DNA protection:

Observation concerned with number of apoptotic cells (brown colored spots) in the gastric mucosa are presented in fig. The number of apoptotic cells (brown spots) was significantly greater in the gastric mucosa of ethanol induced group compared to the control group ($p<0.001$). We found there is a significant decrease in the number of apoptotic cells in groups rats pre-treated with MRME (100mg/kg and 200mg/kg doses) compared to control group($p<0.001$). Thus, the assay clearly indicates the DNA protecting ability in the gastric mucosa of the MRME extract. The TUNEL numbers of brown spots among different groups of are given in figure 4.16.

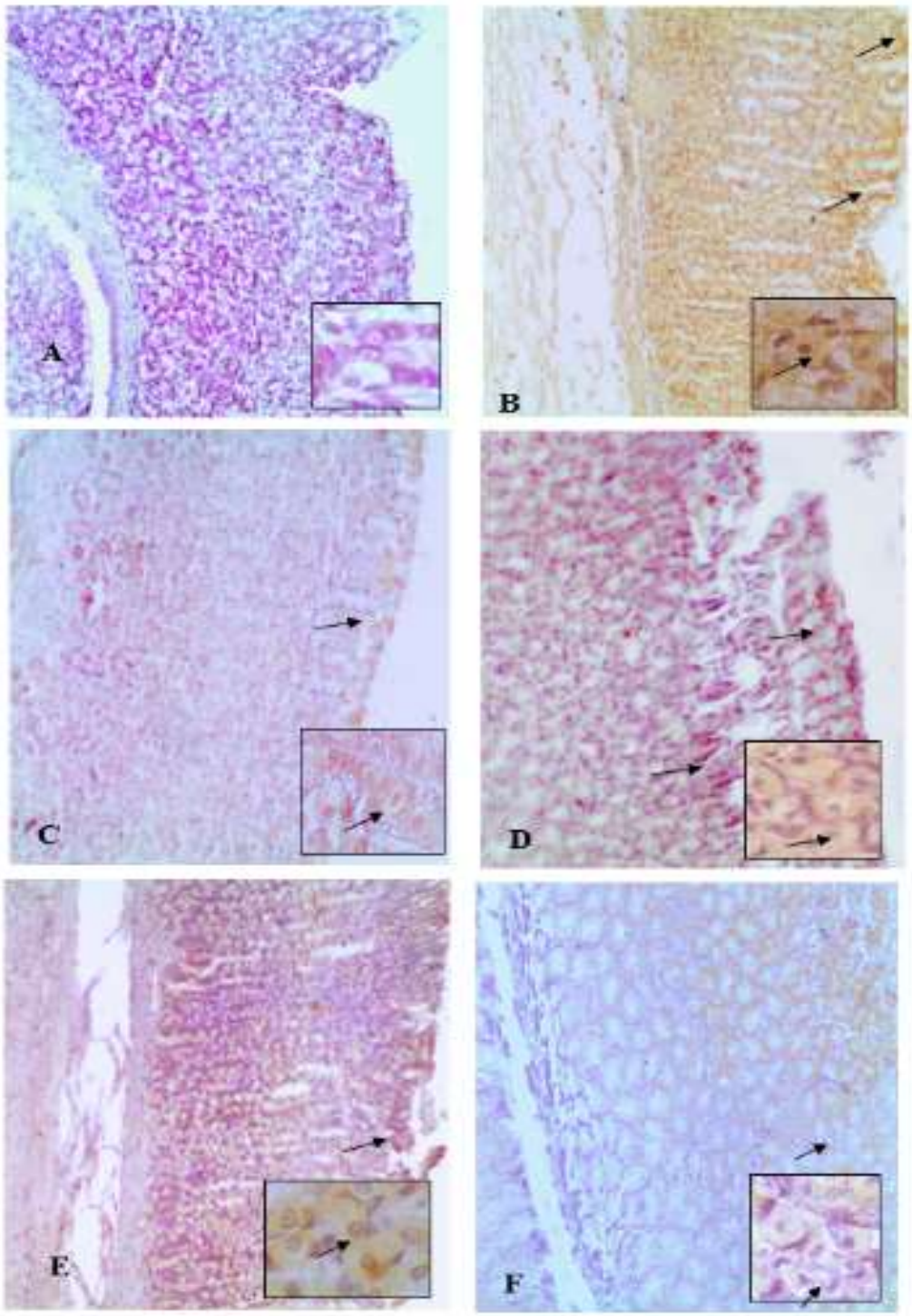


Fig. 4.12 (I) Caspase-3 expression: Representative photomicrographs showing BAX immunohistochemically staining magnification 10X and in each image small box (40X). (A)Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Immunopositive areas are brown in colour showed by black arrows indicate immunopositive cells.

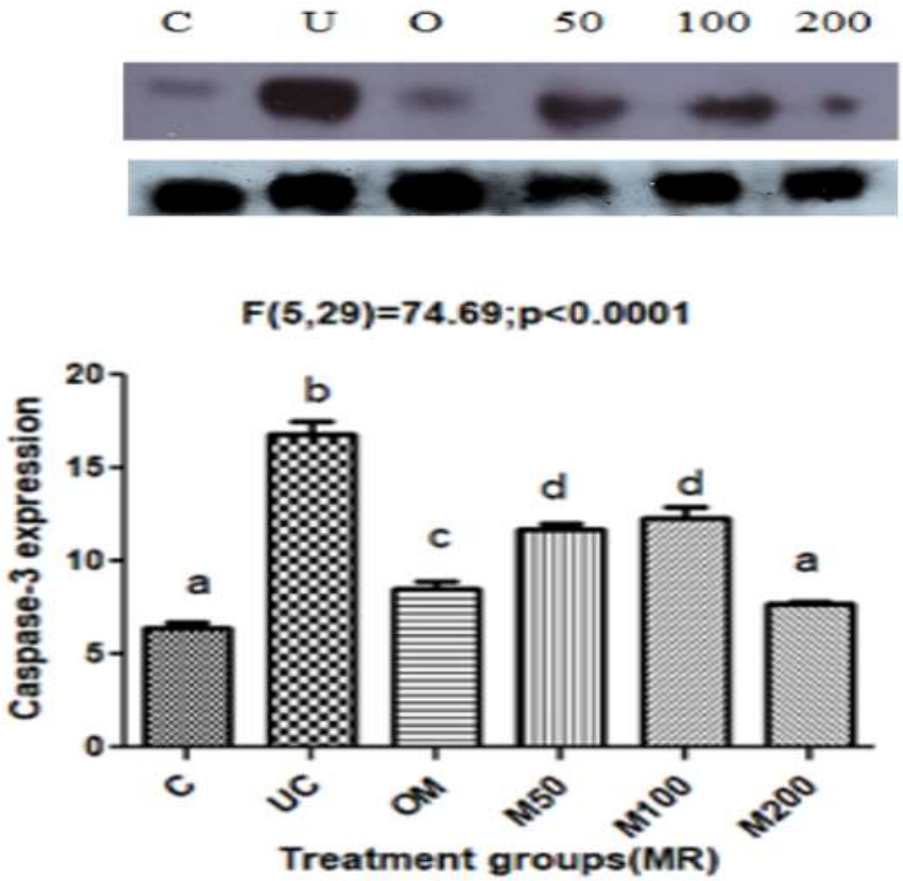


Fig. 4.12(II) Caspase-3 expression; The graph represents semi-quantification of Caspase-3 with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (M50) ;(M100) ;(M200) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Representative western blot image is also presented herewith. Statistical comparison was performed using one-way ANOVA followed by Tukey ‘s multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.**

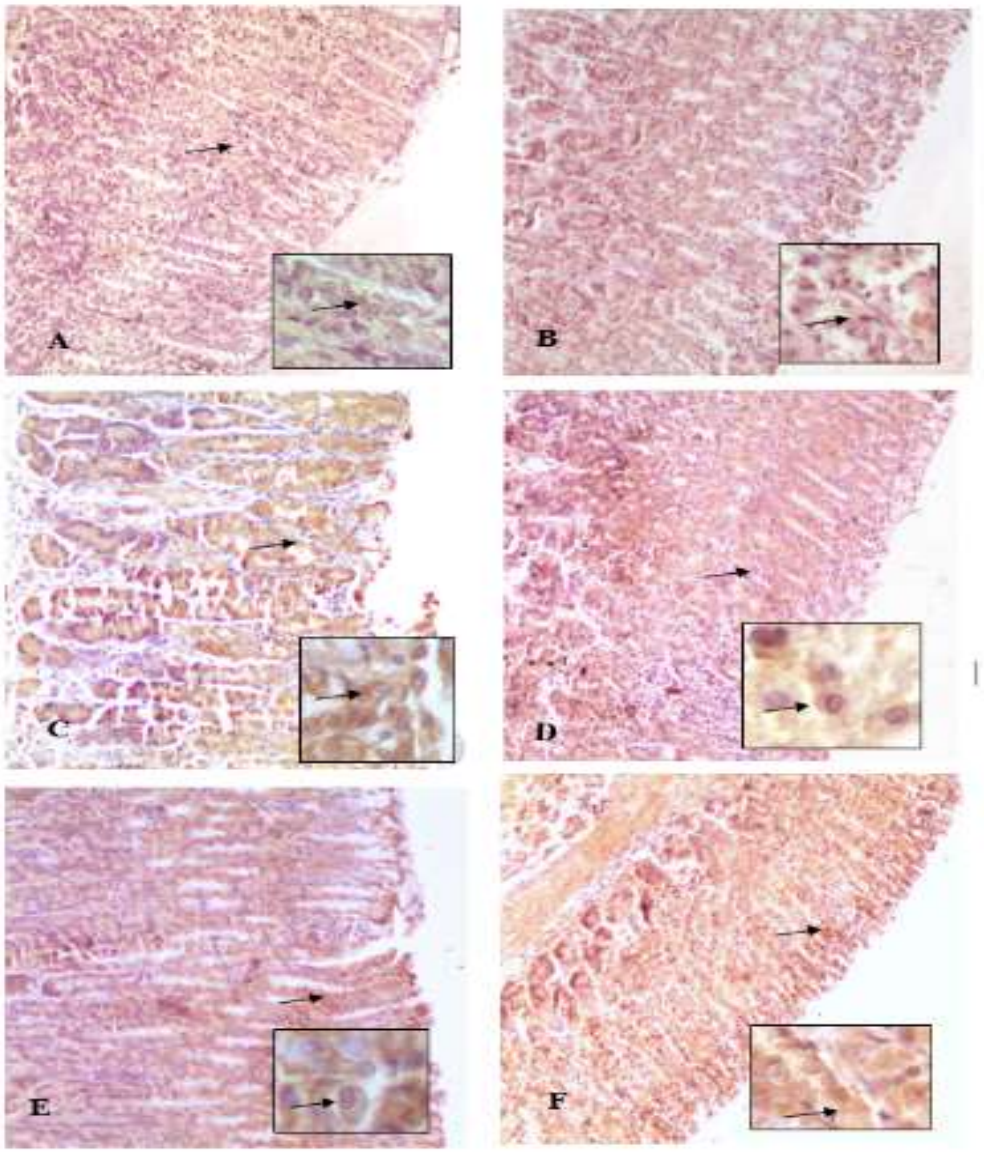


Fig. 4.13 (I) HSP70 expression: Representative photomicrographs showing HSP70 immunohistochemically staining magnification 10X and in each image small box (40X). (A)Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Immunopositive areas are brown in colour showed by black arrows indicate immunopositive cells.

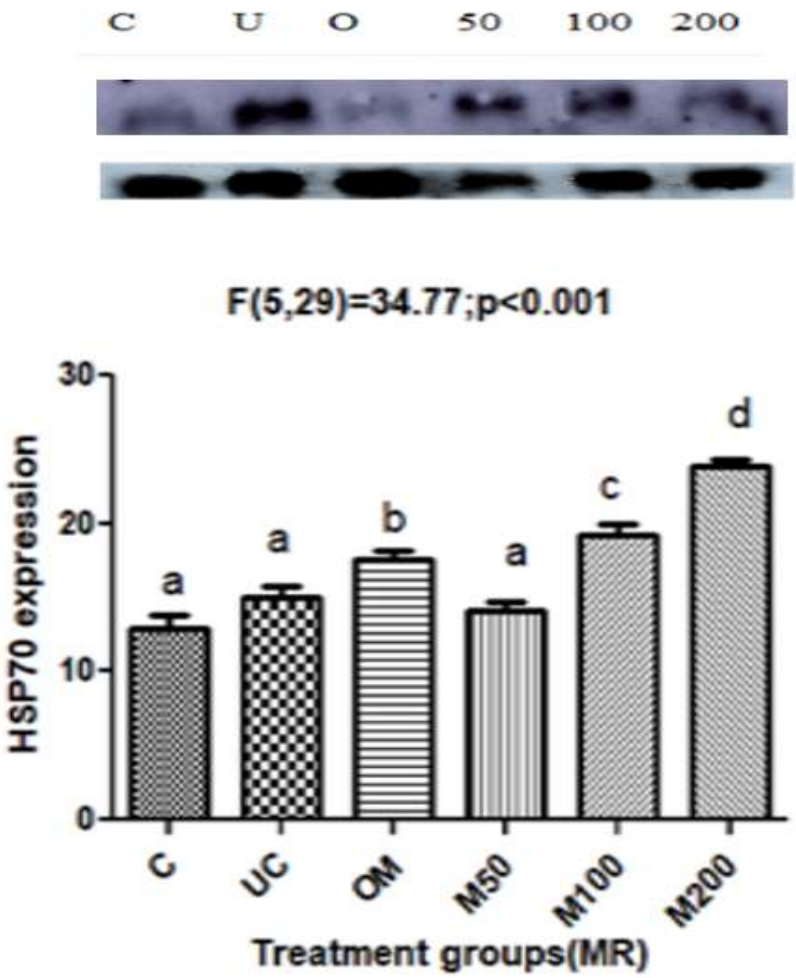


Fig. 4.13 (II) HSP70 expression; The graph represents semi-quantification of HSP70 with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (M50) ;(M100) ;(M200) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Representative western blot image is also presented herewith. Statistical comparison was performed using one-way ANOVA followed by Tukey 's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.**

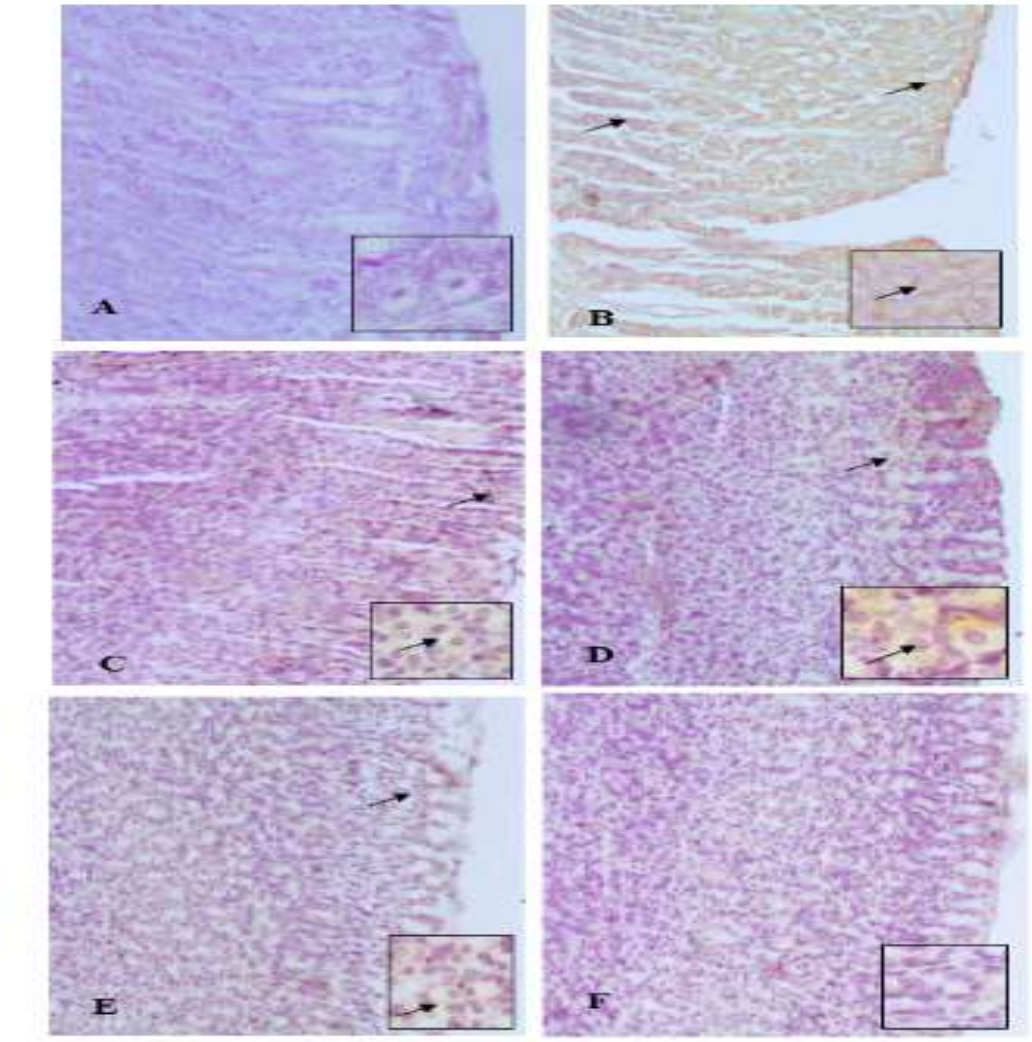


Fig. 4.14 (I) TNF-α expression: Representative photomicrographs showing **TNF-α** immunohistochemically staining magnification 10X and in each image small box (40X). (A)Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with MRME at the dose of 50,100 & 200mg/kg. Immunopositive areas are brown in colour showed by black arrows indicate immunopositive cells.

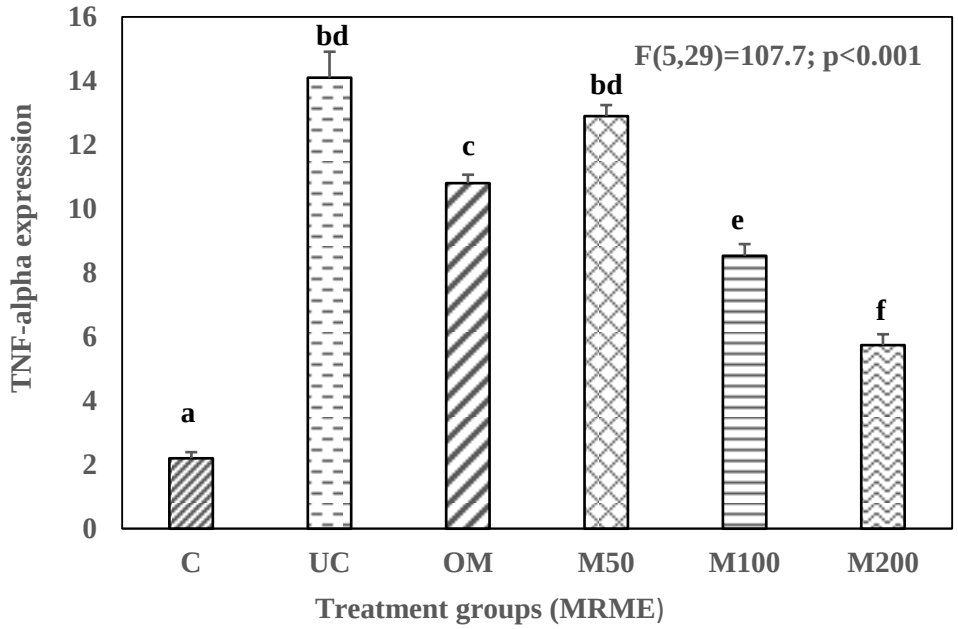


Fig. 4.14 (II) TNF-α expression; The graph represents semi-quantification of HSP70 with 10X IHC stained area analyzed by Image J, (1Normal control group, (2) Ulcer group, (3) Omez, (4) ;(5) ;(6); group pretreated with MRME at the dose of 50,100 & 200mg/kg. Statistical comparison was performed using one-way ANOVA followed by Tukey ‘s multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.**

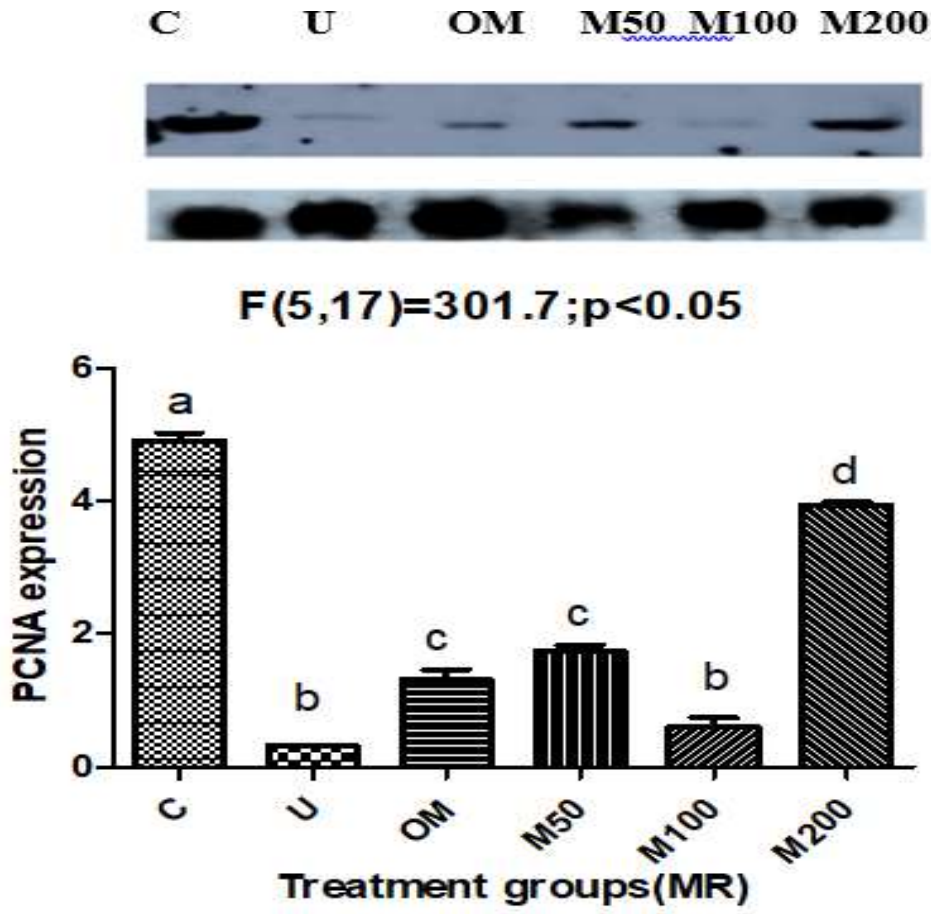


Fig. 4.15(I) PCNA expression (Western blot): The graph represents semi-quantification of PCNA with immunoblot image analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (G200); (G400); (G600) groups pretreated with MRMME at the dose of 50, 100 & 200mg/kg. Statistical comparison was performed using one-way ANOVA followed by Tukey ‘s multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.** Data are expressed as mean ± S.E with five rats in each group.

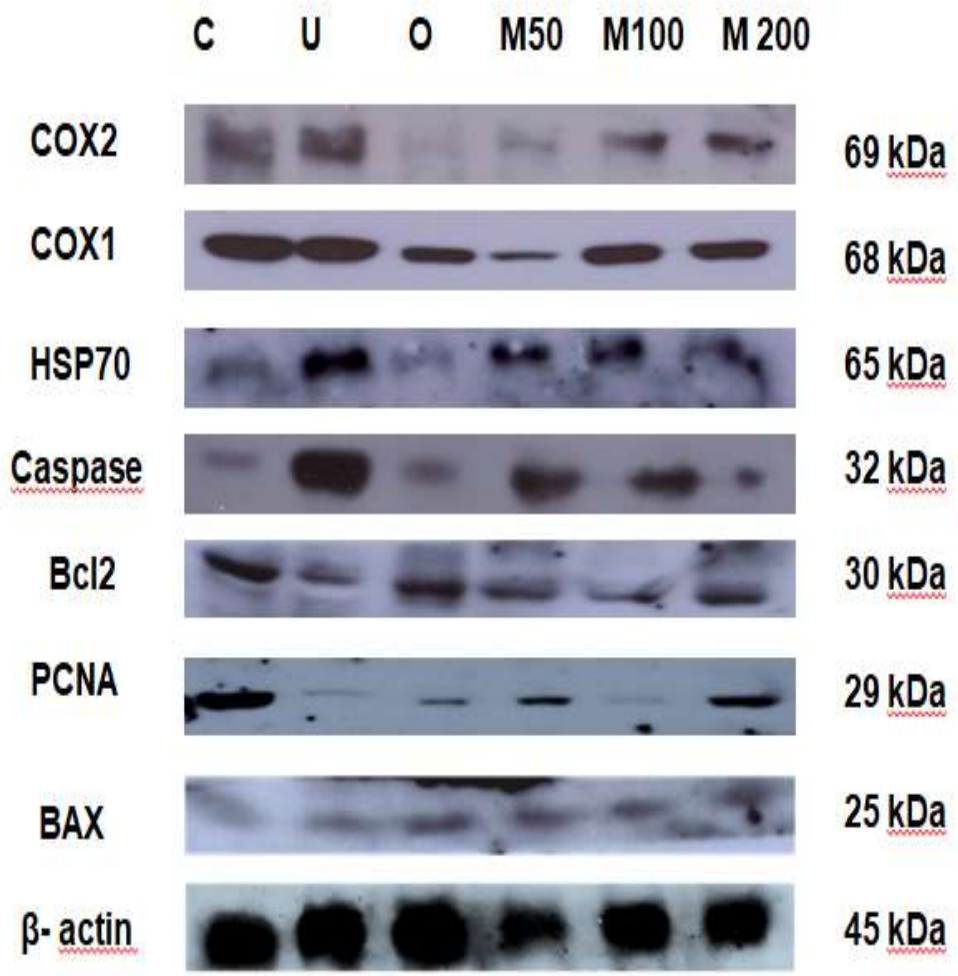
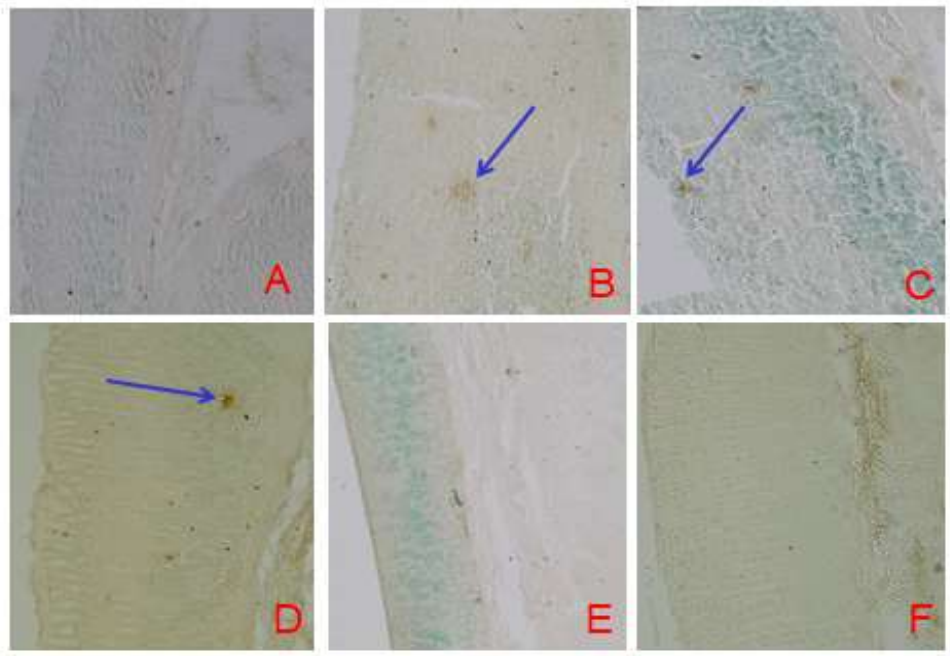


Fig. 4.15 (II) Western blot expression of various cellular regulatory proteins: (C) Normal control group,(U)Ulcer group (O) Omez: (M50); (M100); (M200); group pretreated with MRME at the dose of 50, 100, & 200mg/kg; The MRME has effectively exerted gastro cell protective through maintenance/up regulation COX-I, COX-II(responsible for PGE & NO synthesis, gastric flow), BCL2(anti-apoptotic), PCNA(DNA replication & cell cycle regulation) and HSP70(prevent cell from aberrant aggregation and refolding of miss folded proteins), effectively down regulated apoptotic proteins(BAX and Caspase-3).



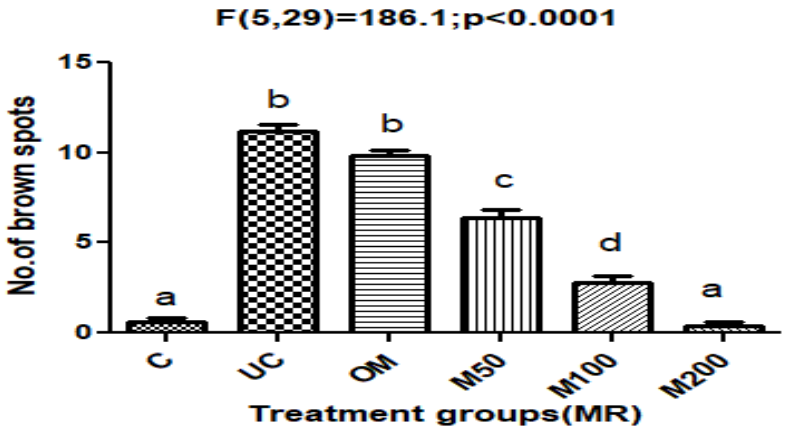


Fig. 4.16 Tunnel Assay for DNA damage activity: (A) Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with MRME at the doses of 50,100 & 200mg/kg, DNA damage (brown spot) recorded in ulcer, omez and 50 mg, while significant ($p<0.001$) DNA protection was observed in 100 & 200 mg MRME doses. In graph (C)Normal control group, (UC)Ulcer group, (OM) Omez, (M50) ;(M100) ;(M200); group pretreated with MRME at the dose of 50,100 & 200mg/kg. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and while similar letters are not statistically significant.

B. *Garcinia lanceifolia* Roxb.

RESULTS:

4.7 Phytochemical analysis of methanol extract of *G. lanceifolia* (GLME)

A total of 2918 and 1082 named and unnamed compounds were listed in the methanolic extracts of *G. lanceifolia* for positive and negative ionization mode respectively. Out of which the unnamed and repeated prediction were deleted from the prediction list. Further the filter is limited to full match in the mzCloud Best Match; it was edged to more than 145 compounds for the methanolic extracts of *G. lanceifolia* in positive and negative ionization mode. These compounds were searched in the Pub Chem compounds to identify the plant derived metabolites and biological properties with respect other *Garcinia* spp. 19 compounds of *G. lanceifolia* methanolic extracts are identified as most probable plant metabolites in the species (Table 2). These compounds were grouped as amino acids (5-Aminolevulinic acid, L-Glutamic acid, L-Dopa, Leucine, L-Cystine, L-Serine, L-Glutamic acid and 3-Hydroxyanthranilic acid), organic acids (Pyrrole-2-carboxylic acid, L-(-)-Malic acid and Citric acid), fatty acids and their derivatives (Hexadecanamide, oleamide and 16-Hydroxyhexadecanoic acid), flavonoids (Vitexin, (1S)-1,5-Anhydro-2-O-(6-deoxy-α-L-mannopyranosyl)-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-6-yl]-D-glucitol, Scutellarin, Luteolin) and courmarin (4-Hydroxycoumarin)(Table 2). Henceforth, *G. lanceifolia* methanol fruit extract was found to be a rich source bioactive phytochemicals.

Table 4.2 Identification of the most abundant phytocompounds present in methanol leaf extract of *G. lanceifolia* by HR-LC-MS

Sl. No.	Phytocompounds identified	RT (min)	Molecular weight	Annotation: mzCloud	Ionization state	Nature of compound
1.	5-Aminolevulinic acid	0.911	131.0585	Full match	[M+H]+	Amino acid
2.	L-Glutamic acid	0.986	147.0534	Full match	[M+H]+	Amino acid
3.	Pyrrole-2-carboxylic acid	1.01	111.0322	Full match	[M+H]+	Organic acid
4.	L-Dopa	1.012	197.0691	Full match	[M+H]+	Amino acid
5.	Leucine	1.019	131.0949	Full match	[M+H]+	Amino acids
6.	L-Cystine	2.271	240.0242	Full match	[M+H]+	Amino acids
7.	Vitexin	11.61	432.1046	Full match	[M+H]+	Flavonoid
8.	(1S)-1,5-Anhydro-2-O-(6-deoxy-α-L-mannopyranosyl)-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-6-yl]-D-glucitol	11.951	578.163	Full match	[M+H]+	Biflavonoid
9.	Scutellarin	12.437	462.0799	Full match	[M+H]+	Flavonoid
10.	4-Hydroxycoumarin	20.254	162.0318	Full match	[M+H]+	Coumarin
11.	Hexadecanamide	22.95	255.2556	Full match	[M+H]+	Fatty acid
12.	Oleamide	23.154	281.2708	Full match	[M+H]+	Fatty acid
13.	L-Serine	29.099	105.0427	Full match	[M+H]+	Amino acid
14.	L-Glutamic acid	0.968	147.053	Full match	[M-H]-	Amino acid
15.	L-(-)-Malic acid	1.023	134.0214	Full match	[M-H]-	Organic acid
16.	Citric acid	1.235	192.0267	Full match	[M-H]-	Organic acid
17.	3-Hydroxyanthranilic acid	7.865	153.0425	Full match	[M-H]-	Amino acid intermediate
18.	Luteolin	14.92	286.0476	Full match	[M-H]-	Flavonoid
19.	16-Hydroxyhexadecanoic acid	22.888	272.2349	Full match	[M-H]-	Fatty acid

4.8 *in vivo* acute toxicity of Garcinia lenceifolia methanolic extract

The results of acute toxicity test of GLME revealed that oral administration of a single dose(1000, 2000, and 3000mg/kg) showed signs of morbidity or mortality in treated animals during the14days.We recorded the behavioral changes in rats(Table 4.3).The rats exposed showed behavioral changes like lethargy, at all the doses given during the treatment period; however, food and water consumption were decreased compared to the control group, the weight loss of the treated groups was lower compared to the control group with significant difference, the histopathological analysis of the liver, kidney,and spleen changes were observed in these organs of the control and GLME-treated groups. All animal deaths were recorded during the14 days of observation. Therefore, it is assumed that the LD50 of GLME might be less than 1000mg/kg.

Table 4.3: Behavioural response and general appearance of rat treated with single dose of *Garcinia lanceifolia* in acute toxicity study.

OBSERVATIONS	Control	1000 mg/kg	2000 mg/kg	3000 mg/kg
Change in skin colour	No effect	No effect	No effect	No effect
Eye colour change	No effect	No effect	No effect	No effect
Food intake	Normal	Not much intake	Not much intake	Not much intake
General physique	Normal	Nil	Nil	Nil
Diarrhoea	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present
Tremor	Not present	Not present	Not present	Not present
Death	Alive	Death	Death	Death

Table 4.4: Mortality pattern in Wister rats orally administered of methanolic extract of Garcinia lanceifolia fruits by gavage in acute toxicity study.

Experiment	Dose (mg/kg)	Mortality				
		Day (0-3)	Day (4-5)	Day (7-9)	Day (10-12)	Day (13-15)
Control	0	0/5	0/5	0/5	0/5	0/5
G. lanceifolia	1000	0/5	1/5	2/5	5/5	5/5
	2000	0/5	1/5	3/5	5/5	5/5
	3000	0/5	3/5	5/5	5/5	5/5

4.8.1 Histopathological analysis:

Rats in the pretreated groups (1000, 2000, and 3000 mg/kg) and the control group histological sections of their liver, spleen, and kidney were examined. As it can be seen in the Fig. 4.18, microscopic examination of the experimental animals' liver, spleen, and kidney after GLME dosage administration indicated pathological alterations. The following are the specific findings from the three organ sections: when compared to the control group, the liver architecture of the animals that received the GLME doses (1000, 2000, and 3000 mg/kg) (Fig. 4.18(I) B-D) was significantly different from the control (Fig. 4.18(I) A), and signs of apoptosis, hepatocyte apoptosis, and a few pores are recorded around the hepatic sinusoid (focal necrosis) were seen. Fig. 4.18(II) A-D showed the kidney histological examination. In the control groups, normal glomeruli, tubules, and interstitium were observed (4.18 (II) A). In the groups receiving GLME treatments, renal tubular epithelial cells showed signs of vacuolar degeneration and interstitial edema; mesangial cell proliferation was recorded; the renal glomerulus underwent diminished; and the renal interstitium showed signs of inflammatory cell infiltration (4.18 (II), In spleen, rats treated with GLME (4.18 (III) B-D) all three doses (1000,2000 & 3000 mg/kg) displayed nuclear aggregations and localized necrosis,

whereas the rats from control group displayed normal red and white in the spleen architecture (4.18 (III) A).

4.9 Sub-acute toxicity

For sub-acute toxicity, rats were divided into four groups (n=5 animals each) and treated with control, 50, 100 and 200 mg/kg/day for 28 days. No death was recorded in the experimental sets of control, 50, mg/kg100 mg/kg and 200mg/kg dosage group as shown in the Table 4.5.

4.9.1General physical observations

No observable changes in the physical appearances and behavioural responses were observed in all the rats from treated groups all the rats showed no changes at all when compared with the control group.

Table 4.5: Behavioural response and general appearance of male rat treated with repeated dose of *Garcinia lanceifolia* in sub-acute toxicity study.

Observations	Control	50 mg/kg	100 mg/kg	200 mg/kg
Change in skin colour	No effect	No effect	No effect	No effect
Eye colour change	No effect	No effect	No effect	No effect
Food intake	Normal	No effect	No Effect	No Effect
General physique	Normal	Normal	Normal	Weakness
Diarrhoea	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present
Tremor	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive

Table 4.6: Mortality pattern in Wister rats orally administered of methanolic extract of *Garcinia lanceifolia* fruits by gavage in sub-acute toxicity study.

Experiment	Dose(mg/kg)	Mortality	
		Day-0	Day-28
Control	0	0/5	0/5
Garcinia lanceifolia	50	0/5	0/5
	100	0/5	0/5
	200	0/5	0/5

4.9.2 Histological changes in organs *in vivo* sub-acute toxicity

For sub-acute toxicity, rats were divided into four groups (n=5) and treated with control, 50, 100 and 200 mg/kg/day for 28 days. No death was recorded in the experimental sets of control, 50, mg/kg, 100 mg/kg and 200 mg/kg dosage groups.

After histological & microscopic examination, results shown in the figure 4.18. When compared the liver, kidneys, and spleen of the animals treated with GLME (50, 100, and 200 mg/kg) to the control group, histological examination revealed no observable abnormalities in the structural integrity of any of the animals' examined organs.

4.10 Gastric lesion evaluation, gastric pH, ulcer index and ulcer preventive index:

The rat group administered with absolute alcohol (5 ml/kg) showed a distinct mark of injury and lesions in the gastric mucosa. Hemorrhagic lesions and uneven corroded mucosal ulcerations were observed in the gastric tissue (Fig. 4.17 B) compared to the normal control group (Fig. 4.17 A) and positive control group rats (Omeprazole pre-treated (20 mg/kg) (Fig. 4.17 C). Rats pre-treated with GLME exhibited significant marked protection GLME200 and GLME400 in a dose-dependent manner (Fig. 4.17 D & E). However, a very mild symptom of mucosal injury was observed in GLME200, while GLME400 showed no mucosal lesion compared to ulcer control

groups (Fig. 4.17 B, D, and E). Conversely, at a higher dose of GLME600 again, gastric lesions and mucosal injury were observed (Fig. 4.17 F).

Rats pre-treated with absolute alcohol (5ml/kg) exhibited a significant decrease ($p<0.001$) in pH value (3.5) compared to the normal control group (Fig. 4.20 A). Similarly, there was a marked increase in the total acidity in ethanol-induced ulcer groups compared normal control group ($p<0.001$)(Fig 4.20 B). The pre-treated administration of GLME doses 200 and 400 mg/kg significantly enhanced the pH value of gastric juice (5.24-6.09) and simultaneously lowered the total acidity compared to the ethanol-induced rat group ($p<0.001$)(Fig 4.20 A & B).

The absolute ethanol-treated rats exemplified an ulcer index (57.46%). The pre-treatment of rats with GLME at doses of 200–400 mg/kg considerably decreased the GM's ulcer formation ($p<0.001$) and increased the gastric tissue protection (ulcer preventive index, 44–96%) compared to the ulcer control group and positive control omeprazole (ulcer index: 24.62; ulcer preventive index, 43%) ($p<0.001$). Rats pre-treated with GLME at 600mg/kg dose showed ulcer formation (ulcer index 51.22%, preventive index 20.81%). The highest percentage inhibition rate was observed at a dose of 400mg/kg of GLME (Fig. 4.20 C & D).

4.11 Histopathology of GM lesions (H&E) and PAS staining

Microscopic histological examinations with hematoxylin and eosin stains showed a significant marked injury in the gastric mucosa. A deep nurrow loss of surface epithelium and necrosis was observed (Fig. 4.21 B) compared to the normal control group in which no gastric lesion was observed (Fig. 4.21 A) and positive control group omeprazole pretreated group (Fig. 4.21 C). The rat group pretreated with GLME400 showed significant protection compared to the ulcer control group and GLME200 with less superficial gastric lesions (Fig. 4.21 E & 3D). Rats pretreated with 600 mg/kg GLME showed distinct mucosal injury and loss of epithelial cells in microscopic images (Fig. 4.21 F). Overall, tissues that received pre-treatment with GLME400 had comparatively better protection.

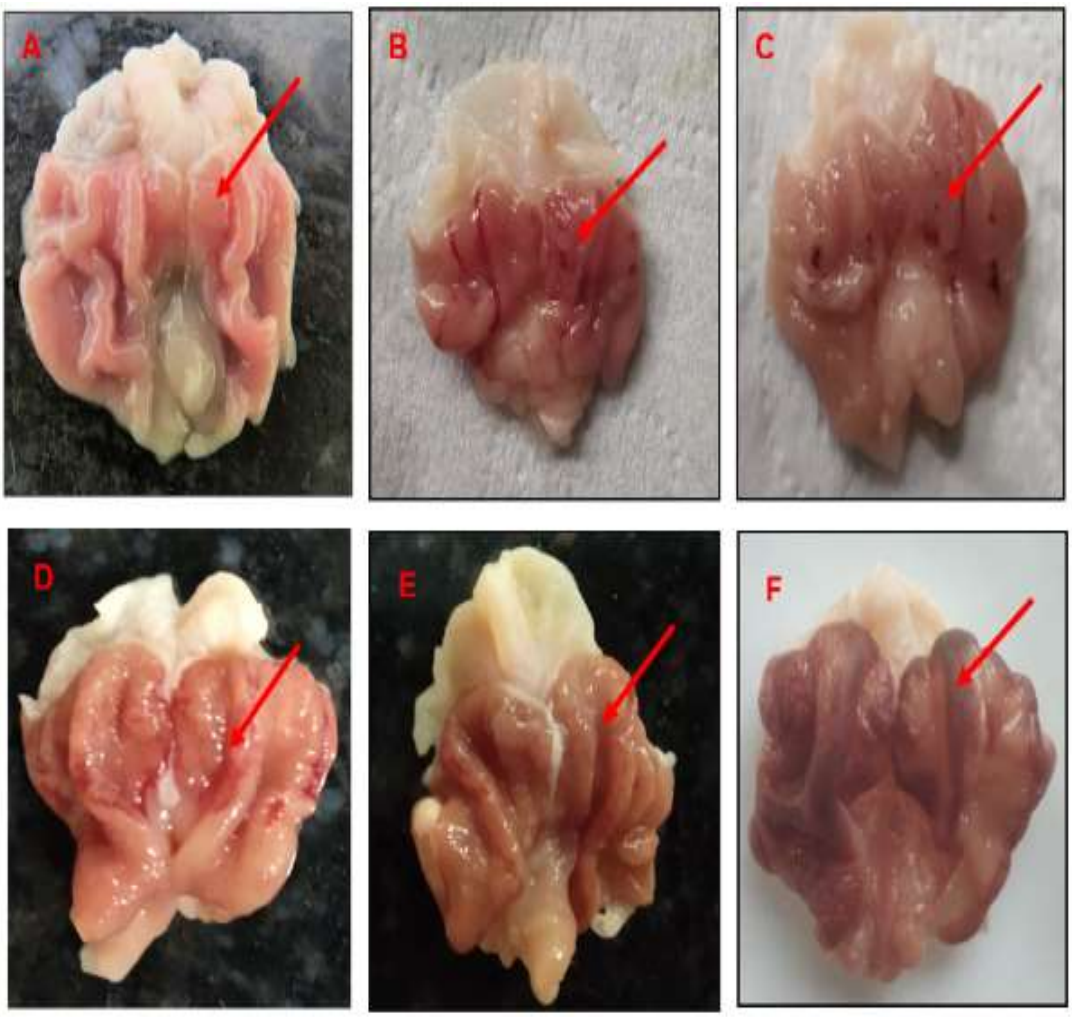


Figure 4.17 Gastro-protective effect of *G. lanceifolia* methanolic fruit extract on gross appearance of the gastric mucosa in Wistar albino rats. (A) Normal control group demonstrated flattening of mucosal folds and normal coloration. (B) Ulcer control group, rats pre-treated with absolute ethanol (5 mL/kg) in exhibited corroded gastric mucosal folds, broad, deep and perceptible haemorrhagic lesions, deep ulcers and Perforations. (C) Reference group, rats pre-treated with omeprazole (20 mg/kg) showed mild gastric mucosa injuries with thin ulcers and small perforations compared to ulcer control rats. (D, &E,) Rats pre-treated with 200, & 400 mg/kg, respectively showing the signs of protection and restoration from ulcer formation. (F) Rats pre-treated with 600mg/kg showing deep ulcer, -haemorrhagic lesions, Arrows indicate the normal folding patterns of gastric mucosa in Group-A and E and site of injury in B, C, D and F groups.

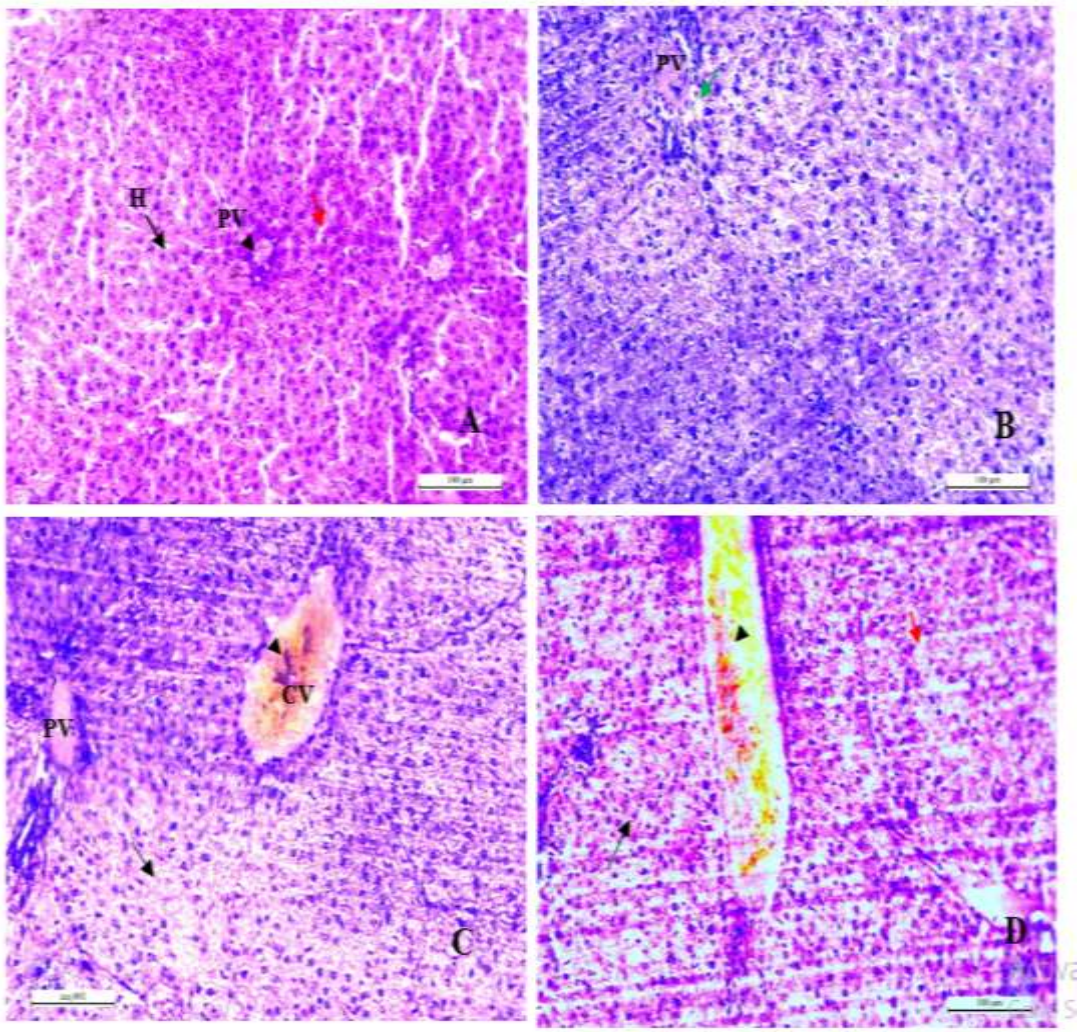


Fig.4.18(I) Acute toxicity assay of *G. lancefolia* methanol fruit extract (GLME): Histology of liver (H&E, 20x) of control and GLME-treated animals. (A) section of liver from control group animal revealed normal architecture with hepatocytes arranged around the central vein; hepatic cells with granulated cytoplasm, (B) section from 1000mg/kg showed inflammation around central vein, (C) section from 2000mg/kg group exhibited disturbed architecture of hepatocytes and hepatic cells, and central venous congestion of liver lobule, (D) section from 3000mg/kg group showed focal necrosis, granular degeneration of hepatocytes(H), black arrow indicate hepatocytes, black arrow head indicates central vein(CV), red arrow indicates sinusoid(S),green arrow head show inflammation surrounding central vein.

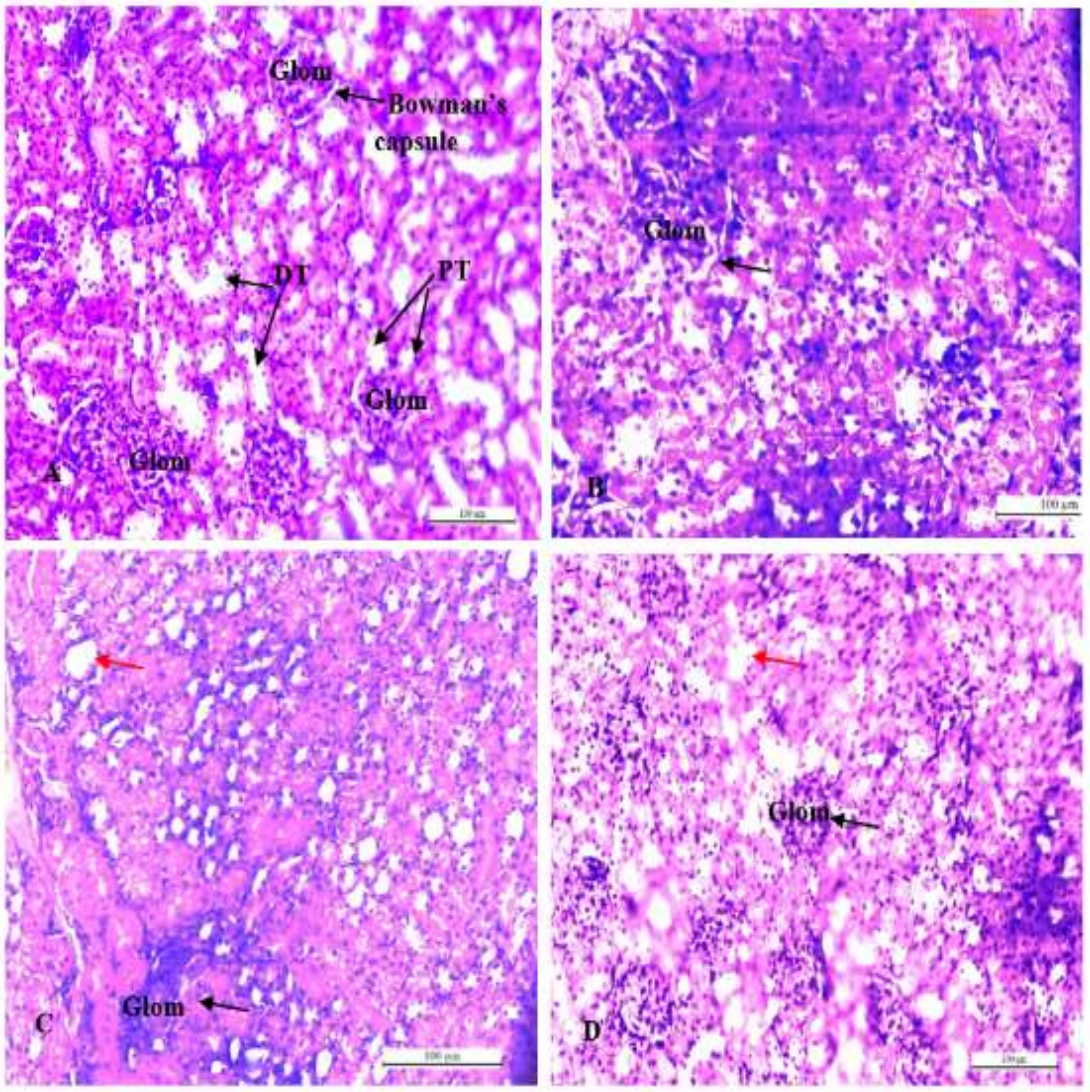
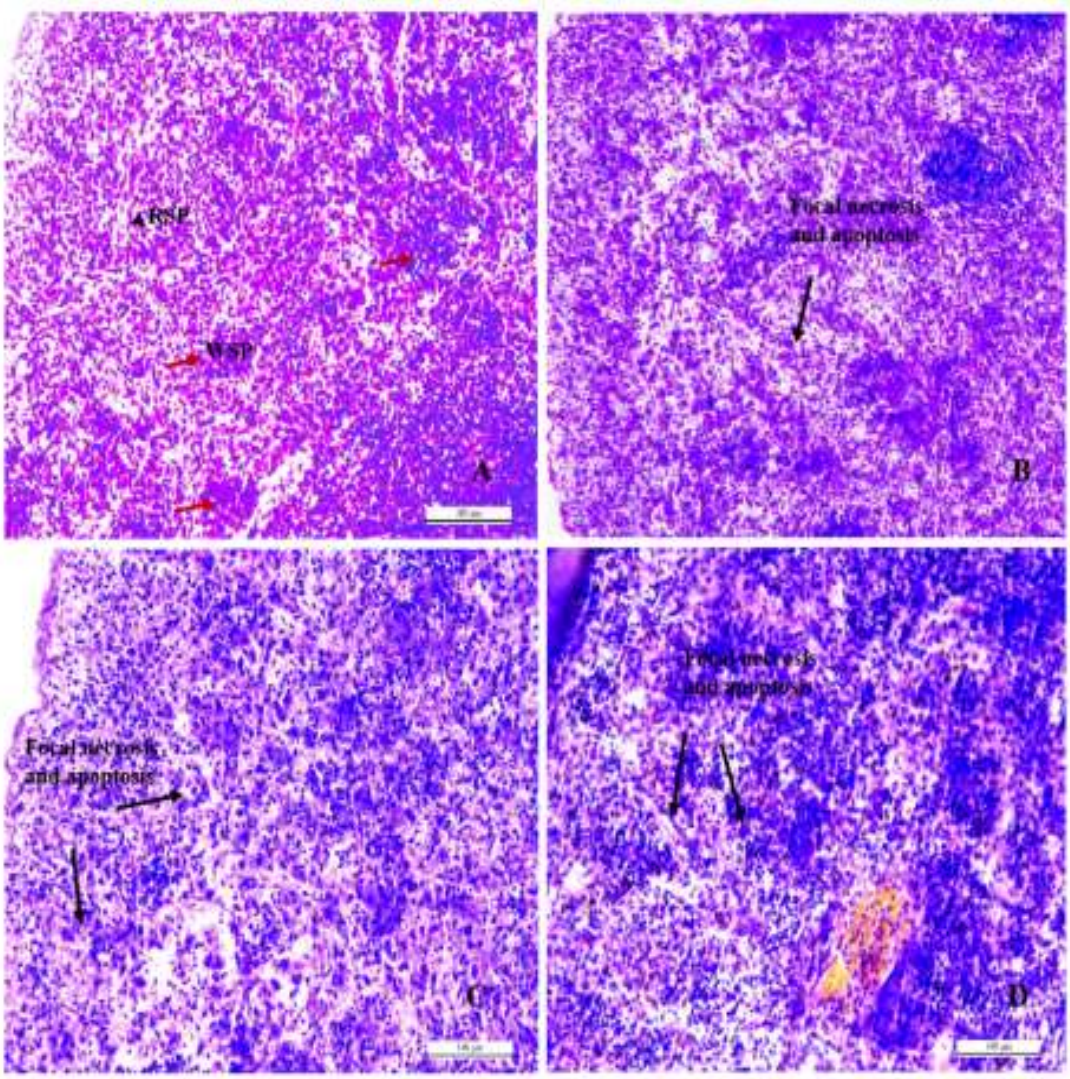


Fig 4.18(II). Acute toxicity assay of *G. lancefolia* methanol fruit extract (GLME): Kidney histology at 20x resolution. All the groups section was stained with H&E and observed the glomerulus which is the most effective area of the kidney for toxicity influence. (A) The normal control group showed the normal appearance of kidney, glomerulus (G), proximal tubule (PT) and distal tubule (DT). Group (B), (C) & (D) the toxicity test group, administered with GLME 1000mg/kg, 2000mg/kg & 3000mg/kg observed, irregular & shrunken shapes of glomerulus, granular degeneration. Black arrows show glomerulus structure in different groups, red arrows indicate tubular apoptosis. (DT-distal tubule, PT-proximal-tubule, Glom-glomerulus).



*F*ig.4.18(III) Acute toxicity assay of *G. lancefolia* methanol fruit extract (GLME): Histology of Spleen (H&E, 20x) of control and GLME-treated animals. (A) Section of spleen from control group animal revealed normal white splenic pulp(WSP), Red splenic pulp(RSP) arranged; (B), (C), & (D) section from 1000mg/kg ,2000mg/kg & 3000mg/kg exhibited, disturbed architecture of RSP & WSP, some nuclear aggregations and focal necrosis, black arrow head indicates (RSP), red arrow indicates (WSP).

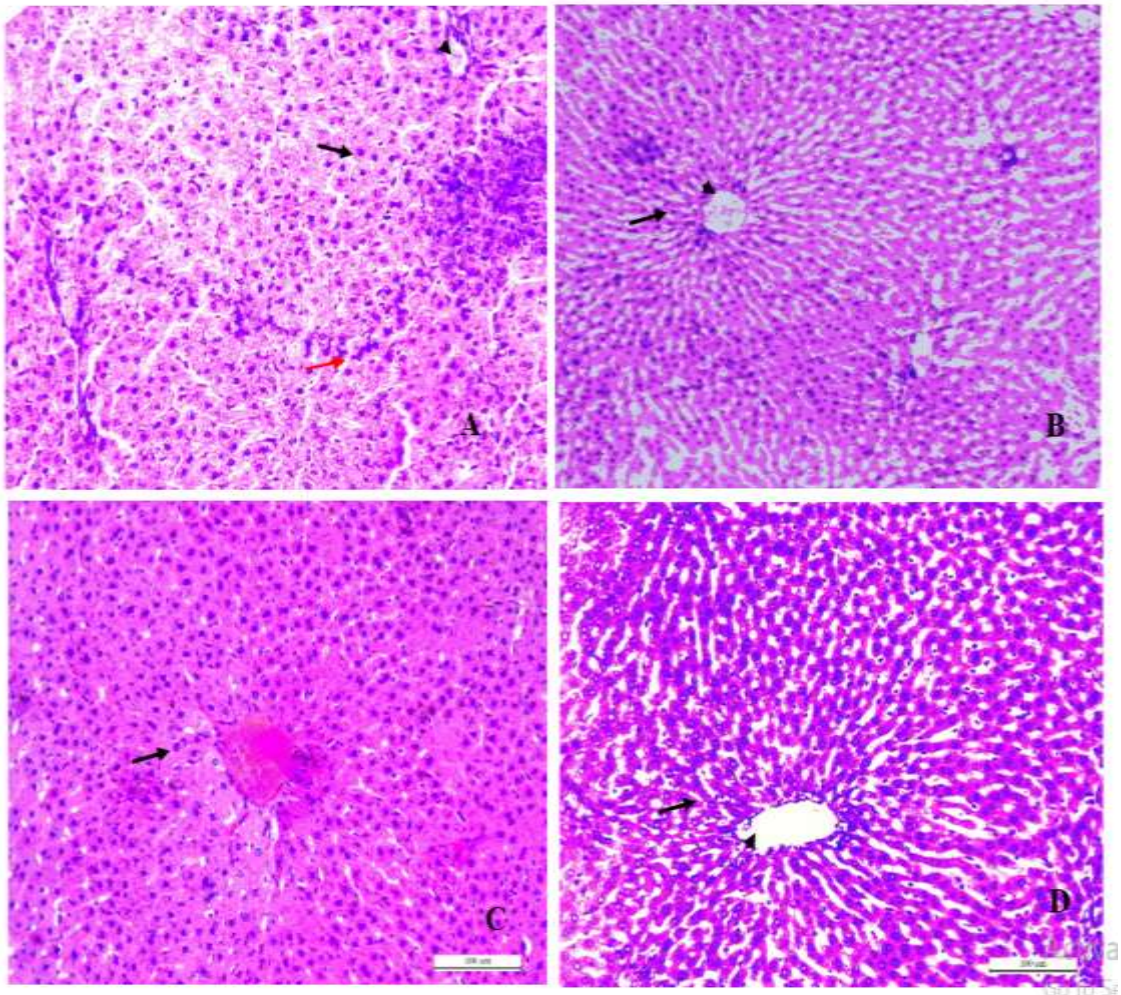


Fig.4.19(I) Sub-Acute toxicity assay of *G. lancefolia* methanol fruit extract (GLME): Histology of liver (H&E, 20x) of control and GLME-treated animals. (A) Section of liver from control animals revealed normal architecture of central vein and hepatic cells with granulated cytoplasm; (B) and (C) liver from GLME (50 and 100mg/kg)-treated animals exhibited likely to normal architecture of hepatocytes and hepatic cells with granulated cytoplasm. The liver section of 200mg/kg group showed mild inflammatory infiltration within the lobule of the liver. (black arrow indicates hepatocytes, black arrow head indicates central vein, red arrow indicates sinusoid).

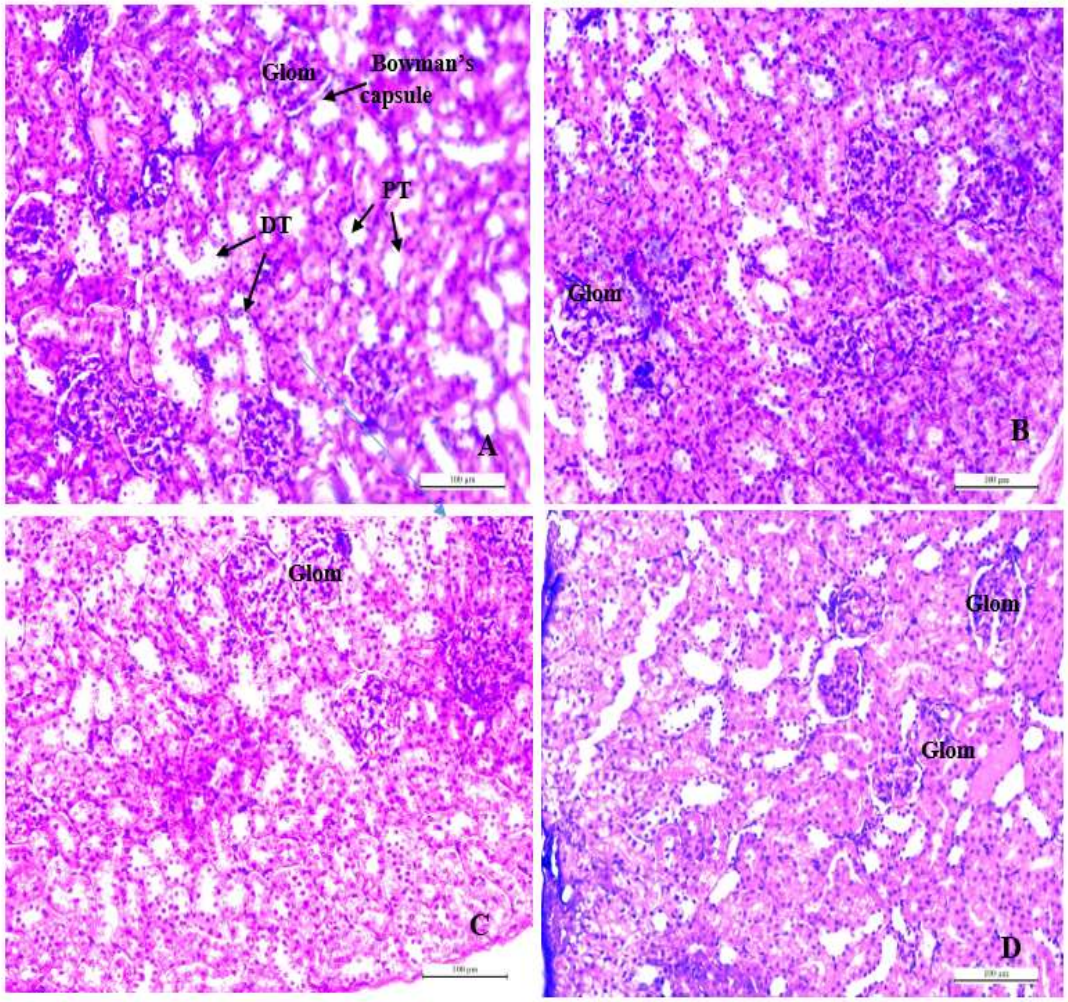


Fig 4.19(II) Sub-acute toxicity assay of *G. lancefolia* methanol fruit extract (GLME) Kidney histology at 20x resolution: control and GLME-treated animals All the groups sections were stained with H&E and observed the glomerulus which is the most effective area of the kidney due to toxicity. (A) The normal control group Showed the normal appearance of kidney, glomerulus (G), proximal tubule (PT) and distal tubule (DT). Group (B), (C) & (D) group, 50mg/kg, 100mg/kg & 200mg/kg showed of glomerulus, DT, PT likely to control group. black arrows show glomerulus structure in different groups, (DT-distal tubule, PT-proximal-tubule, Glom-glomerulus).

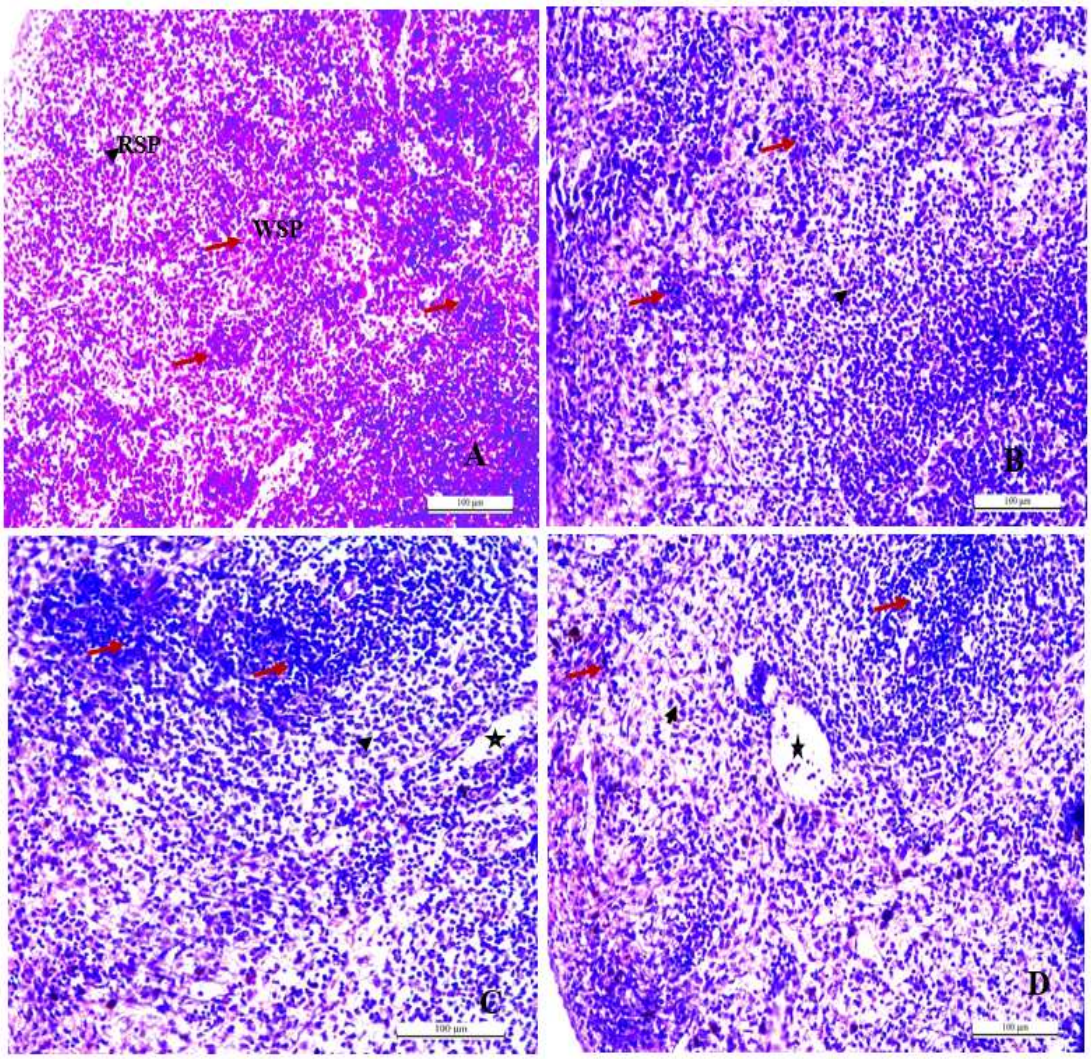


Fig 4.19(III) Sub-acute toxicity assay of *G. lancefolia* methanol fruit extract (GLME): Histology of Spleen (H&E, 20x) of control and GLME-treated animals. (A) Section of spleen from control group animal revealed normal white splenic pulp(WSP), Red splenic pulp(RSP) arranged ;(B), (C) & (D) section from 50mg/kg ,100mg/kg & 200mg/kg exhibited normal architecture of RSP & WSP, vacuolar degeneration is observed in the middle layer of a splenic arteriole from 100 & 200mg/kg group of GLME, black arrow head indicates Red Splenic Pulp(RSP), red arrow indicates (WSP), star indicates vacuolar degeneration.

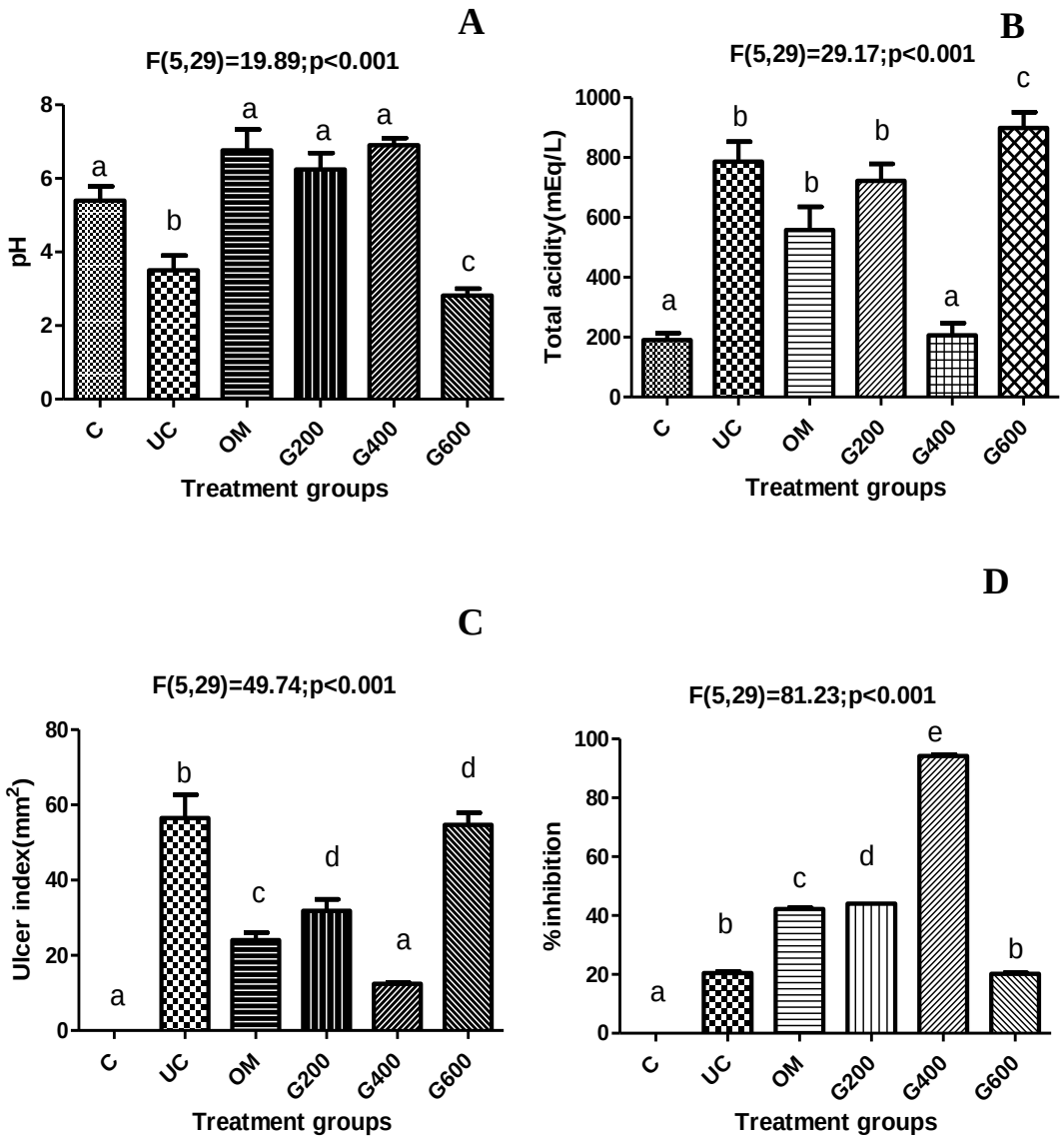


Fig. 4.20 Effects of *G. lanceifolia* methanol fruit rind extract (GLME) on: (A) pH, (B)total acidity (mEq/L), (C) Ulcer index and (D) % inhibition (C)Normal control group, (UC)Ulcer group (OM) Omez:(G200) ;(G400) ;(G600) group pretreated with MRME at the dose of 200,400 & 600mg/kg, Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and while similar letters are not statistically significant.

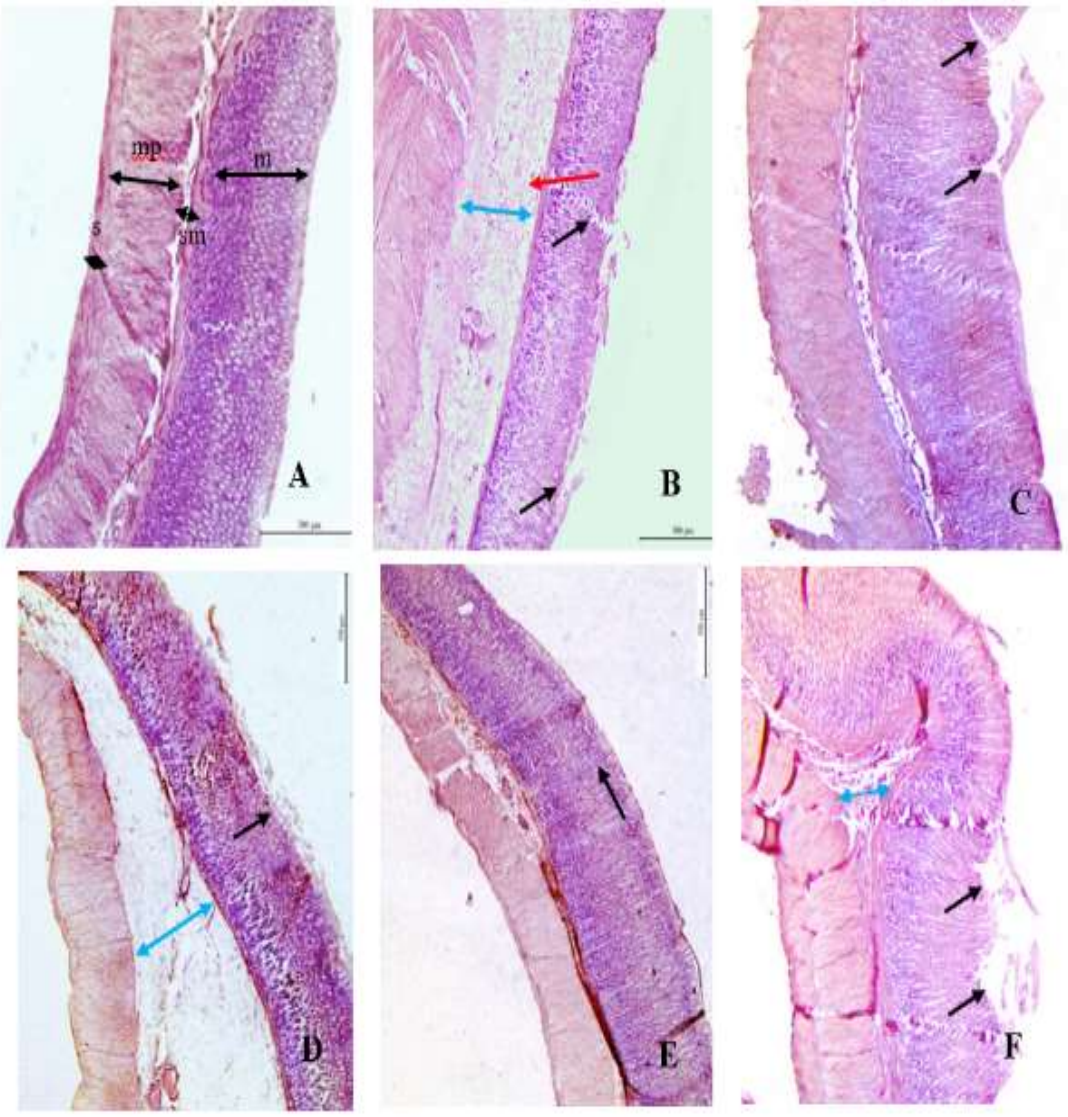


Fig. 4.21(I) Histology of gastric mucosal damage in rats (**5X**). In the normal control group (A), no disruption to the surface epithelium was observed, with normal architecture of stomach with mucosa(m), submucosa(sm), muscularis externa(me) & serosa(s) layer. The ulcer control group (B) has severe disruption to the surface epithelium (black arrows) and necrotic lesions penetrate deeply into mucosa and extensive edema of submucosal layer (blue arrows) and leucocyte infiltration (red arrows) are present. The reference control group (omeprazole, 20 mg/kg) (C), showed mild disruption of the surface epithelium mucosa was present but deep mucosal damage was absent. Reduction of submucosal edema and leucocytes infiltration was seen. Rats received 200 mg/kg of the GLME (D) has moderate disruption of surface epithelium with submucosal edema and leucocytes infiltration of submucosal layer. Rats received 400 mg/kg of the GLME (E) showed no disruption to the surface epithelium. In rats received 600 mg/kg of the GLME (F), there is disruption to the surface epithelium. Reduction of submucosal edema and leucocytes infiltration of the submucosal layer are shown.

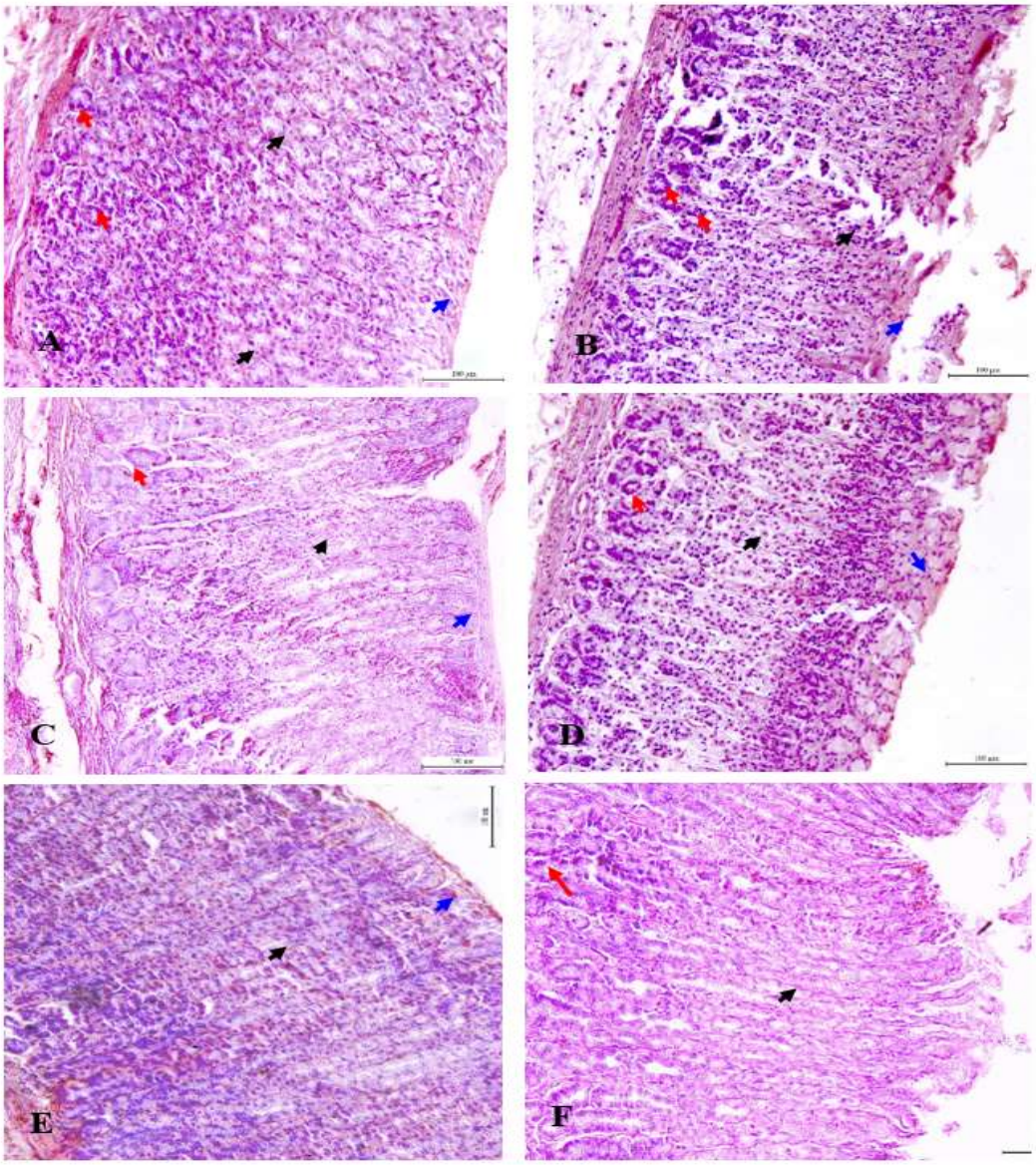


Fig. 4.21(II) Histoly of gastric mucosal damage in rats (**20X**). Histology of normal control group(A) stomach showing the normal structure of glandular mucosa with its normal simple columnar epithelial cover and gastric glands containing chief cell (red arrow) and parietal cells (black arrow), goblet cell (blue arrow). Ulcer control group (B) revealed severe mucosal injury, deep erosions, epithelial damage and glandular disruption. histopathological deformities by loss of stomach architecture, Reference control group(C) (omeprazole showed surface mucosal abrasion with mild haemorrhages., Rats received 200 mg/kg of the GLME (D) showed mild degree of degenerative and desquamative changes of the covering mucosa .Rats received

400mg/kg of GLME(E) showing mild vacuolar degenerative changes within the parietal cells with intact epithelial lining of the gastric gland pits with marked increase of goblet cells, Rats received 600mg/kg group(F) showing necrotic and erosive changes within the covering mucosa.

4.12 Gastric oxidative stress marker (MDA), and NO, cyto-protective antioxidant enzymes

The lipid peroxidation levels (MDA), and antioxidant activities (SOD and GST) were examined in order to investigate the possible role of antioxidant defenses in the gastroprotective effect of *G. lanceifolia*. The data results have found that compared to control group rats, the vehicle group showed a significant ($p < 0.001$) decrease in the stomach mucosal activities of SOD and GSH and an increase in MDA levels (Fig. 4.23 A, B & C). In contrast to the group pre-treatment with methanol extract of *G. lanceifolia* (200, 400, and 600 mg/kg), the GLME ,400mg/kg showed marked significant increase in endogenous SOD and GSH ($p < 0.001$) activities and decreased the MDA level ($p < 0.001$) in the gastric mucosa, respectively, alleviating the oxidative status (Fig 4.23 A, B, & C). The omeprazole group showed significant increase in the level of SOD and GSH and decrease level of MDA as compared to ethanol control group. The maximum effect was observed at the dose of 400mg/kg (Fig 4.23). Ethanol induced ulcers demonstrated a significant decrease in stomach nitric oxide (NO) levels when compared to the control group ($p<0.001$). While GLME pretreated with 600 mg/kg dose showed significant ($p<0.001$) restoration NO levels suggesting *G. lanceifolia* fruit anti-ulcer potential (Fig. 4.23D).

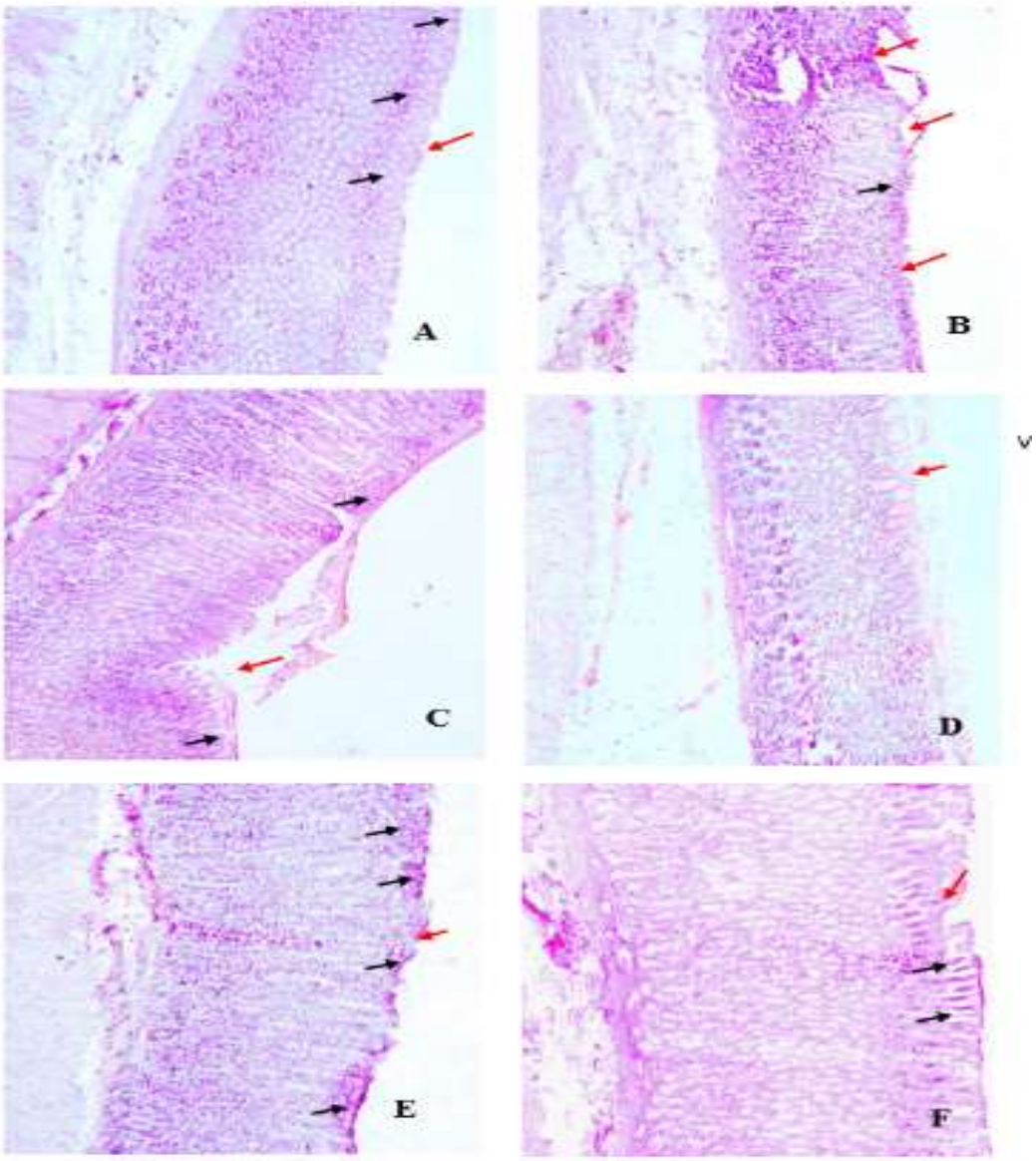


Fig. 4.22 Effect of GLME on gastric tissue glycoprotein-PAS staining(10X) of ethanol-induced gastric damage in rats. (A) Control group showed normal goblet cells within the covering gastric mucosa; (B) Ethanol group; (5 mL/kg) showed marked decrease in mucous cells within the gastric mucosa (C) Omprazole, (20 mg/kg)showed increase in mucous cells within the covering gastric mucosa (arrows); (D) GLME, (200 mg/kg)showed decrease in mucous cells; (E) GLME,400 mg/kg) showing marked increase in mucous cells within the gastric mucosa (arrows; (F) GLME,600 mg/kg showed decrease in mucous cells.The black arrows indicate the glycoprotein, which appears as a magenta stain, and the red arrows indicate loss of continuity. (PAS stain 10X).

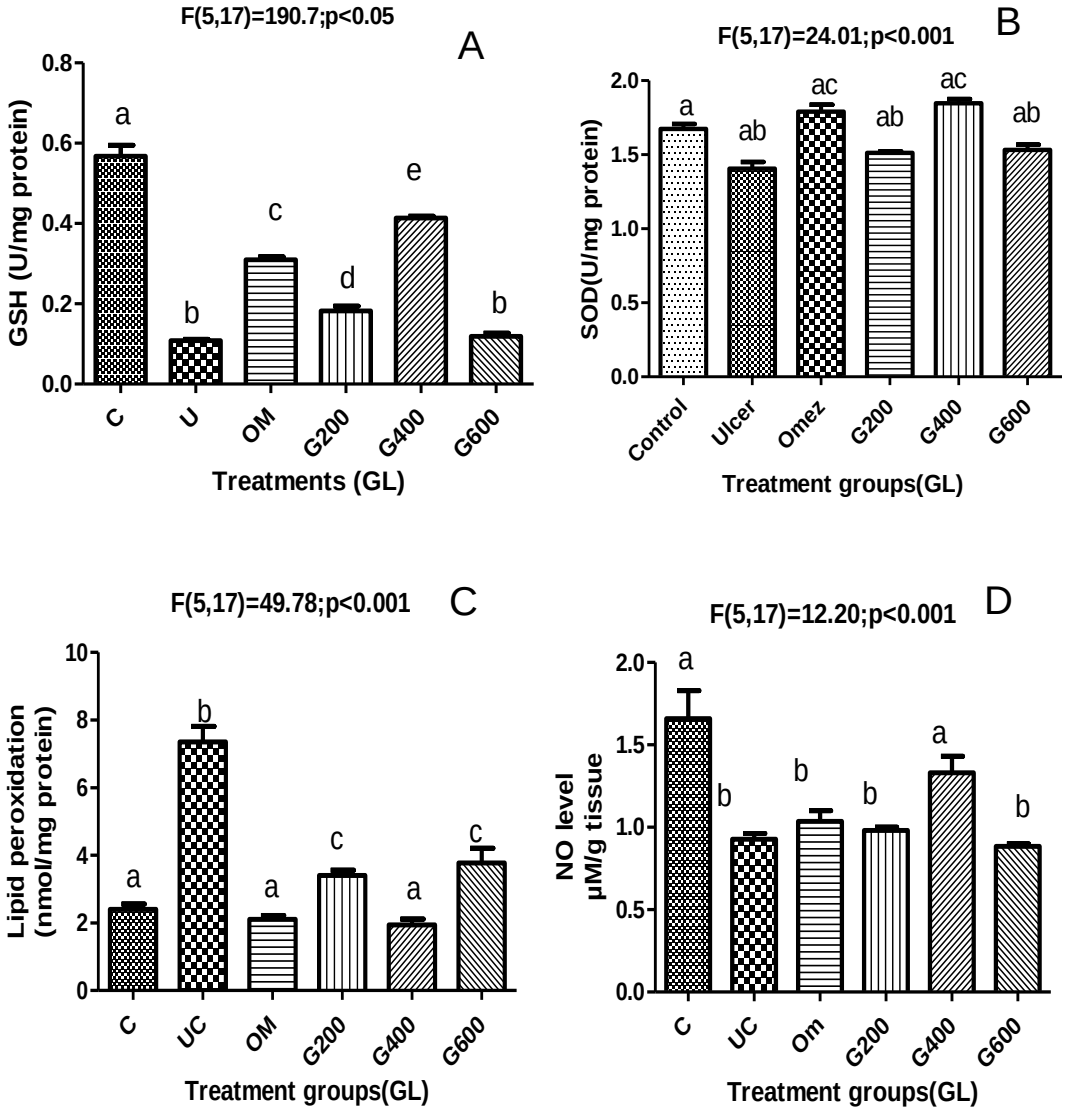


Fig. 4.23 Effects of *G. lanceifolia* methanol fruit rind extract (GLME) on: (A) GST, and (B)SOD antioxidant enzymes scavenging activities (U/mg protein), respectively. (C) Lipid peroxidation (nmol MDA/mg protein) and (D) NO level (µM/g tissue) (C)Normal control group, (UC)Ulcer group (OM) Omez:(G200) ;(G400) ;(G600) group pretreated with MRME at the dose of 200,400 & 600mg/kg, Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and while similar letters are not statistically significant.

4.13 Immunohistochemistry and western blot analysis of expressed proteins in gastric ulcer

Apoptosis, or programmed cell death, has been found as one of the primary causes of stomach ulcer development in earlier research. One key mechanism linked to the regulation of gastric lesions is the inhibition of apoptotic cell death (Konturek et al., 1999). Both in a pathological state and during normal cellular growth, HSP70 is essential for maintaining cell integrity. Through immunohistochemistry study, the behaviour of GLME in the gastric ulcer was further investigated for the expression of HSP70, BCL-2, and BAX proteins in an attempt to find out more about the process by which GLME facilitates the healing of the stomach ulcer. The results are shown in fig. 4.24, 4.25 and 4.26.

4.13.1 BCl2 expression

The immunohistochemical localization results showed up- regulation in expression of the anti-apoptotic BCl2 proteins in the normal control groups of rat. In contrast the expression was significantly down regulated in the ulcer control groups ($p<0.001$). Rat pre-treated with omeprazole showed up-regulated in BCl2 protein expression in the gastric tissue. While GLME dose with 400mg/kg significantly enhanced level of expression of the BCl2 similar that of normal control group ($p<0.001$). Thus middle dose of GLME (400mg/kg) has noticeable protective and anti-apoptotic mode of actions against ethanol induction in the gastric tissue of rats (Fig. 4.24).

4.13.2 BAX expression

The immunohistochemical results showed low level expression of pro-apoptotic Bax protein in normal control group (Fig. 4.25) compared to ulcer control group in which Bax expression was significantly($p<0.001$) higher, indicating ethanol could induce apoptosis in gastric ulcer tissues. Rats pre-treated with omeprazole (reference drug group) and GLME with 400 mg/kg had significantly decreased/down regulated Bax protein expression ($p<0.001$), while pre-treated with GLME (200mg/kg & 600mg/kg) showed up regulation of Bax protein in gastric tissues.

4.13.3 HSP70 expression

In the immunohistochemistry of HSP-70, normal control group (Fig. 4.26A) showed very weak expression while in ulcer group (Fig. 4.26B), the expression of HSP-70 was significantly up regulated ($p<0.001$) group with severe damage. Rats pre-treated with omeprazole and GLME (400mg/kg) (Fig. 4.26C and D) exhibited significant up regulation ($p<0.001$) with moderate/no damage in gastric mucosa, while GLME doses 200 & 400mg/kg showed down-regulation.

4.13.4 Caspase-3 expression

In the study, caspase-3 protein expressions were assessed using western blotting technique to determine role apoptosis marker in gastric induction and amelioration. Figures 4.27 and 4.28 showed the stomach tissues' membranes and representative immunoblots. The ulcer group exhibited significant higher expression levels of caspase-3 in the stomach tissue compared to the control group, which had low expression of caspase-3($p<0.001$). On the other hand, the pre-treated group, which received 400 mg/kg of omez and GLME, had significantly decreased expression of caspase-3($p<0.001$).

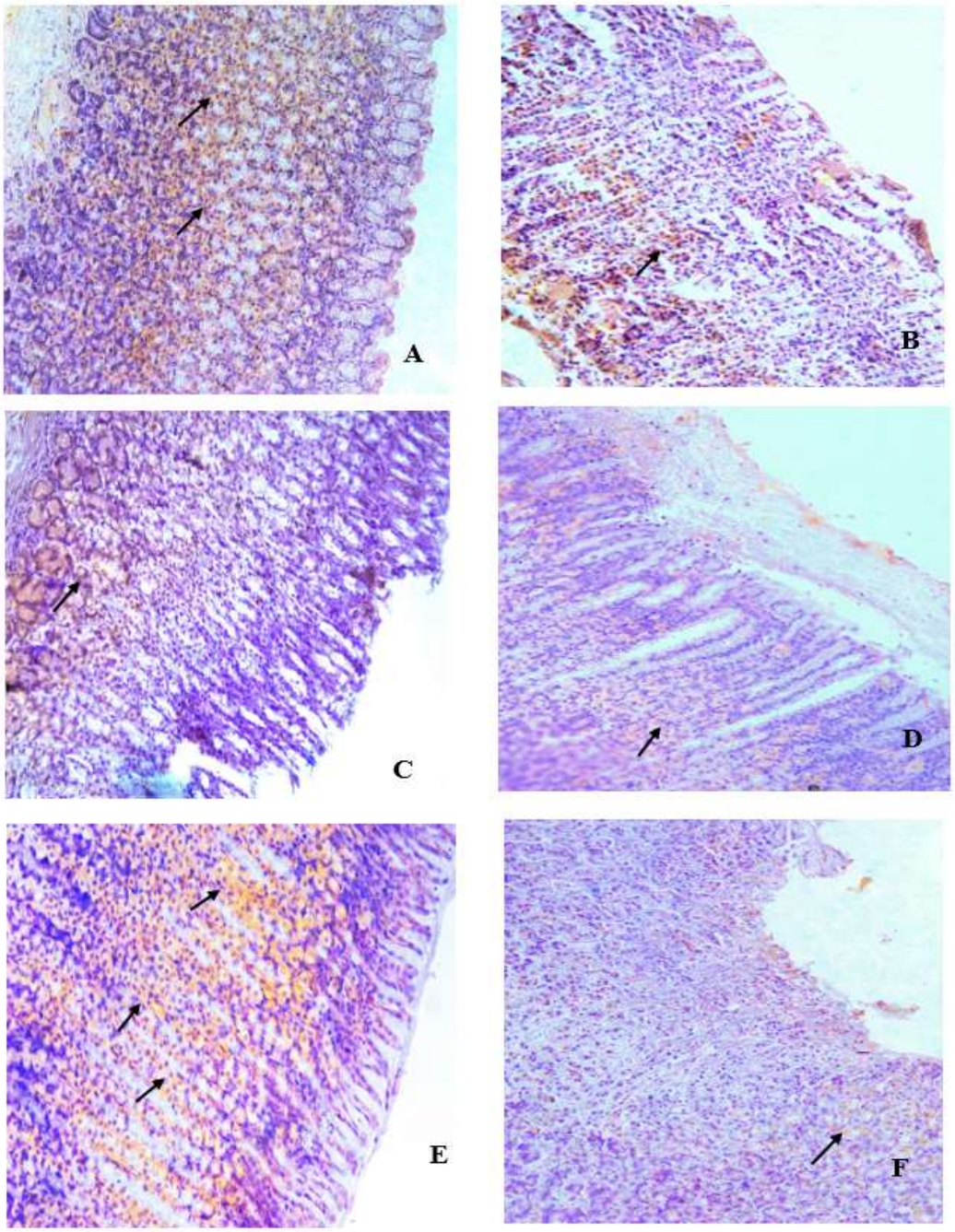


Fig. 4.24 (I) BCL-2 expression: Representative photomicrographs showing BCL-2 immunohistochemically staining magnification 10X. (A)Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with GLME at the dose of 200,400 & 600mg/kg. Immunopositive areas are brown in colour showed by black arrows indicate immunopositive cells.

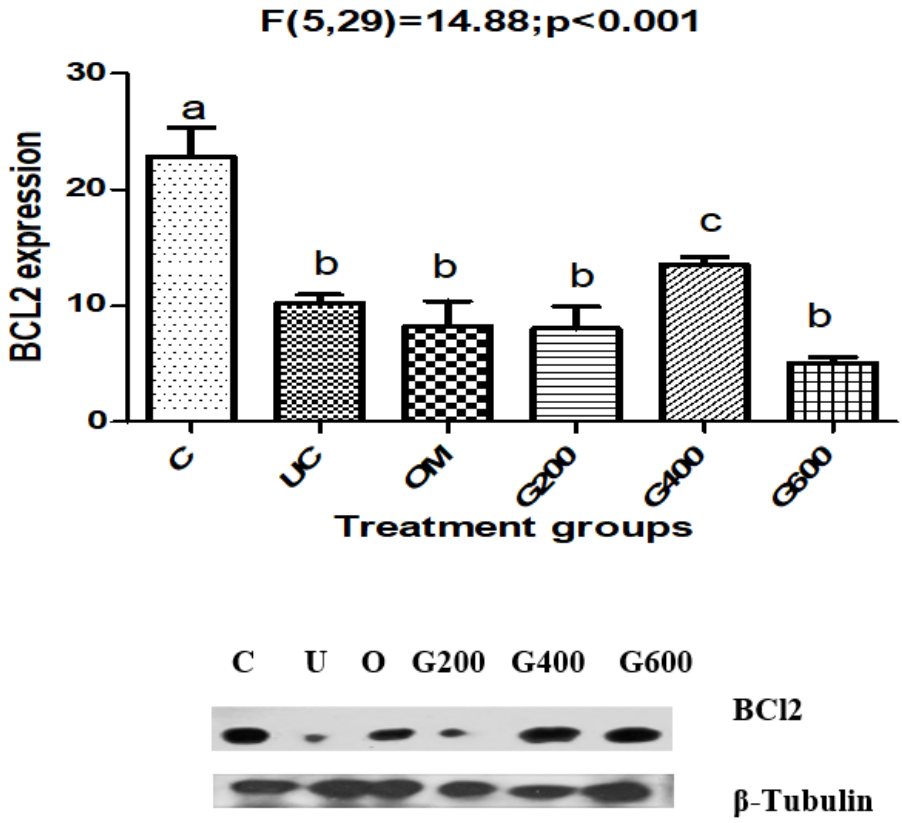


Figure 4.24(II) BCL-2 expression: The graph represents semi-quantification of BCL-2 with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (G200); (G400); (G600) groups pretreated with GLME at the dose of 200, 400 & 600mg/kg. Representative western blot image is also presented herewith. Statistical comparison was performed using one-way ANOVA followed by Tukey 's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.** Data are expressed as mean ± S.E with five rats in each group.

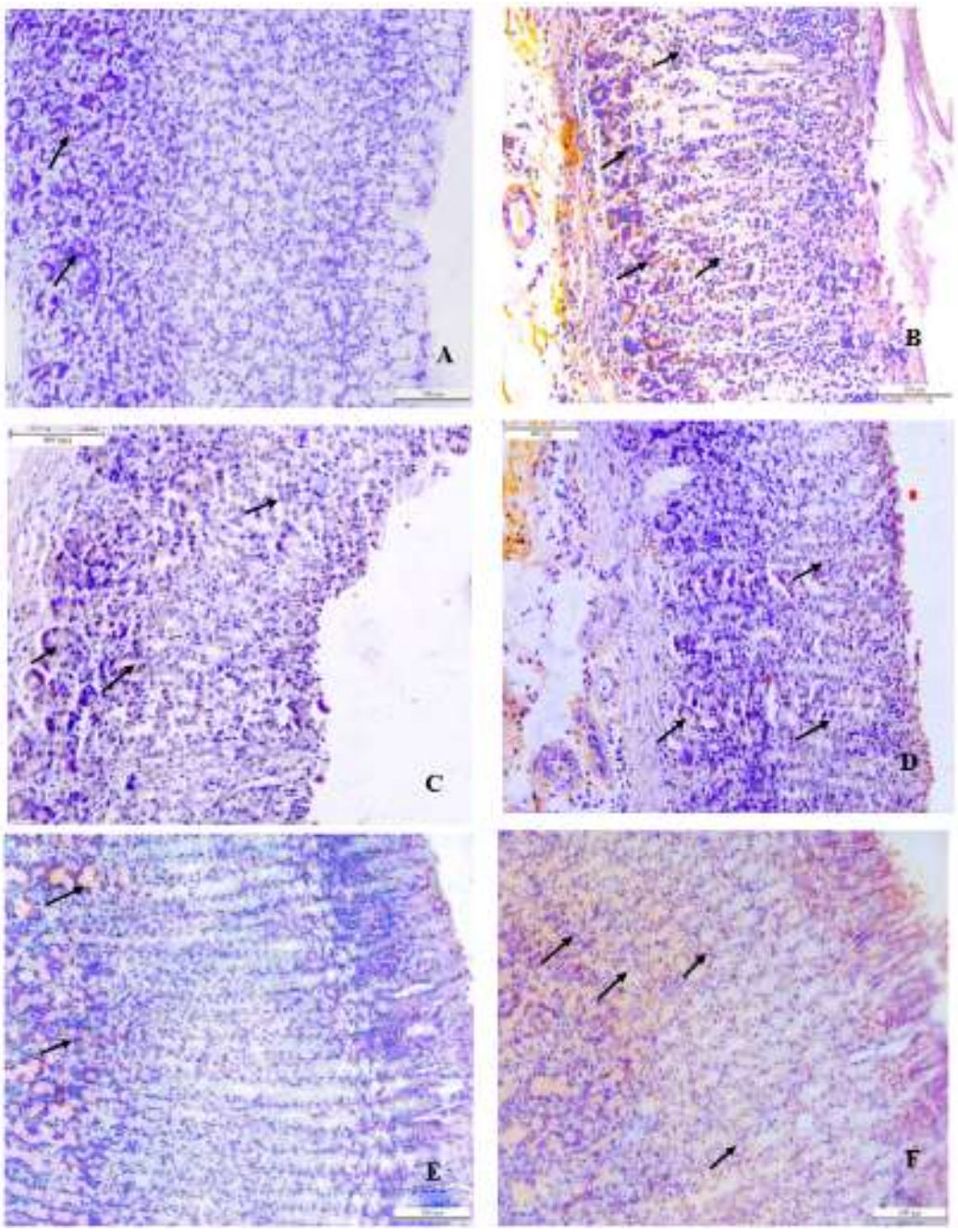


Fig. 4.25 (I) BAX expression: Representative photomicrographs showing BAX immunohistochemically staining magnification 10X. (A)Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with GLME at the dose of 200,400 & 600mg/kg. Immunopositive areas are brown in colour showed by black arrows indicate immunopositive cells.

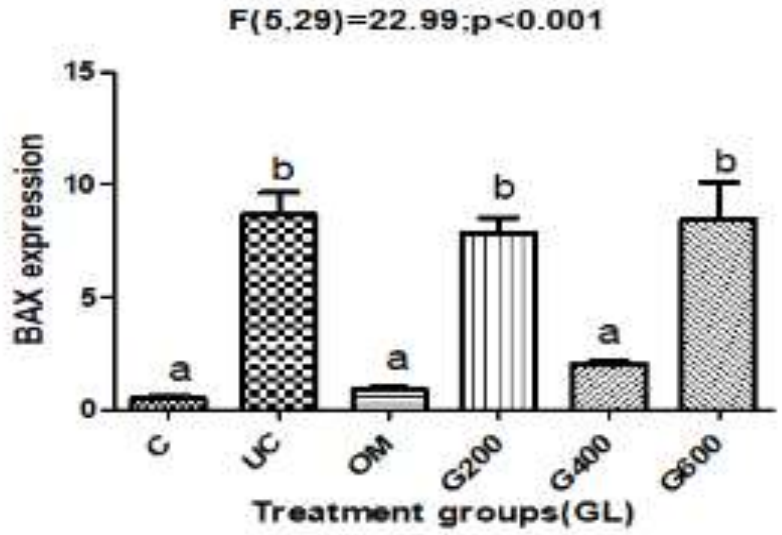


Figure 4.25(II) BAX expression: The graph represents semi-quantification of BAX with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (G200); (G400); (G600) groups pretreated with GLME at the dose of 200, 400 & 600mg/kg. Statistical comparison was performed using one-way ANOVA followed by Tukey 's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.** Data are expressed as mean ± S.E with five rats in each group.

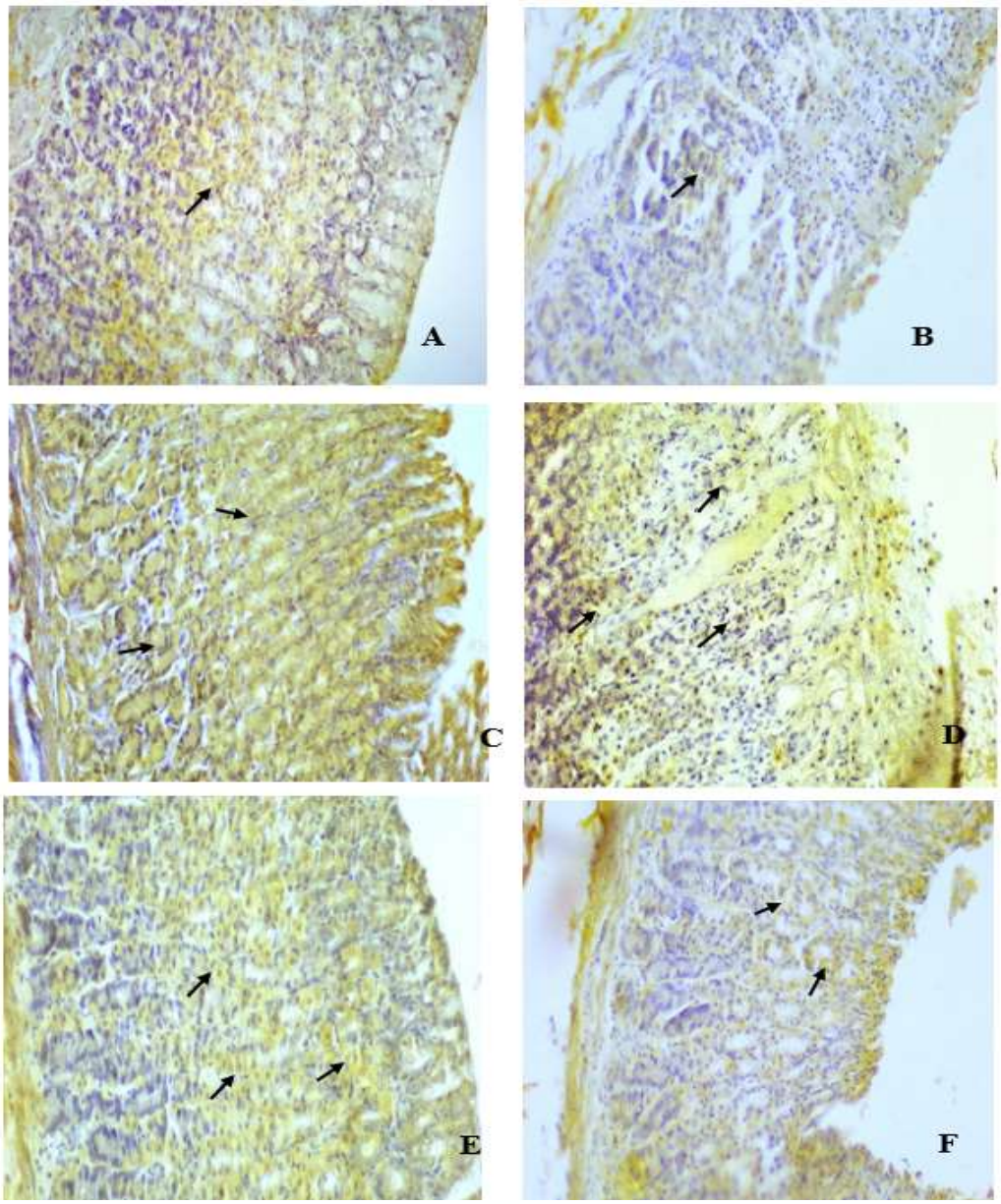


Fig. 4.26 (I) HSP70 expression: Representative photomicrographs showing HSP70-immunohistochemically staining magnification 10X. (A)Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with GLME at the dose of 200, 400 & 600mg/kg. Immunopositive areas are brown in colour showed by black arrows indicate immunopositive cells

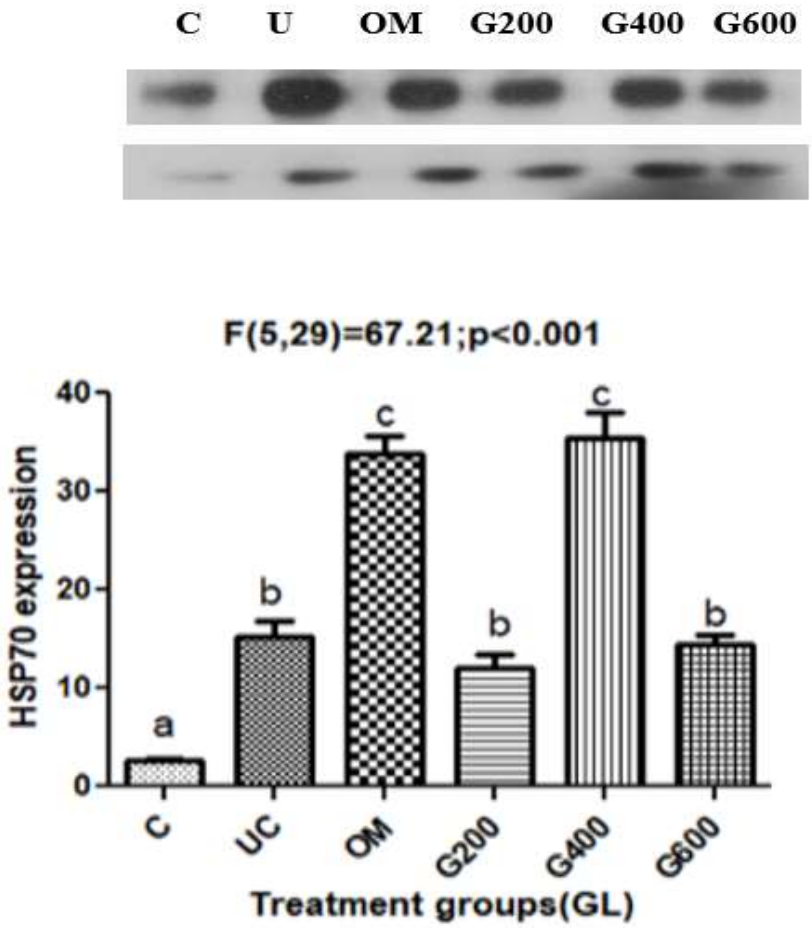


Figure 4.26(II) HSP70 expression: The graph represents semi-quantification of HSP70 with 10X IHC stained area analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (G200); (G400); (G600) groups pretreated with GLME at the dose of 200, 400 & 600mg/kg. Representative western blot image is also presented herewith. Statistical comparison was performed using one-way ANOVA followed by Tukey 's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.** Data are expressed as mean ± S.E with five rats in each group.

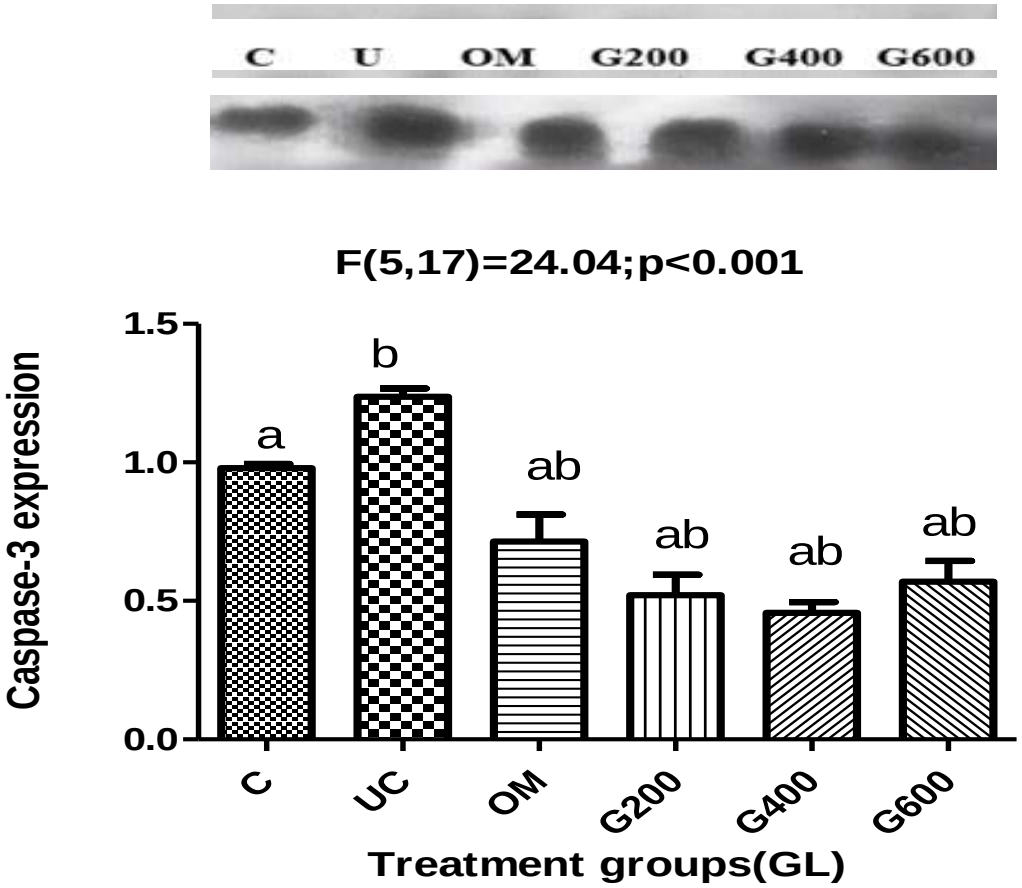


Fig. 4.27 Caspase-3 expression (Western blot): The graph represents semi-quantification of Caspase-3 with immunoblot image analyzed by Image J, (C)Normal control group, (UC)Ulcer group, (OM) Omez, (G200); (G400); (G600) groups pretreated with GLME at the dose of 200, 400 & 600mg/kg. Statistical comparison was performed using one-way ANOVA followed by Tukey 's multiple comparison test. Bar with different letter means statistical difference at $p < 0.001$ and similar letters are not significant**.** Data are expressed as mean ± S.E with five rats in each group.

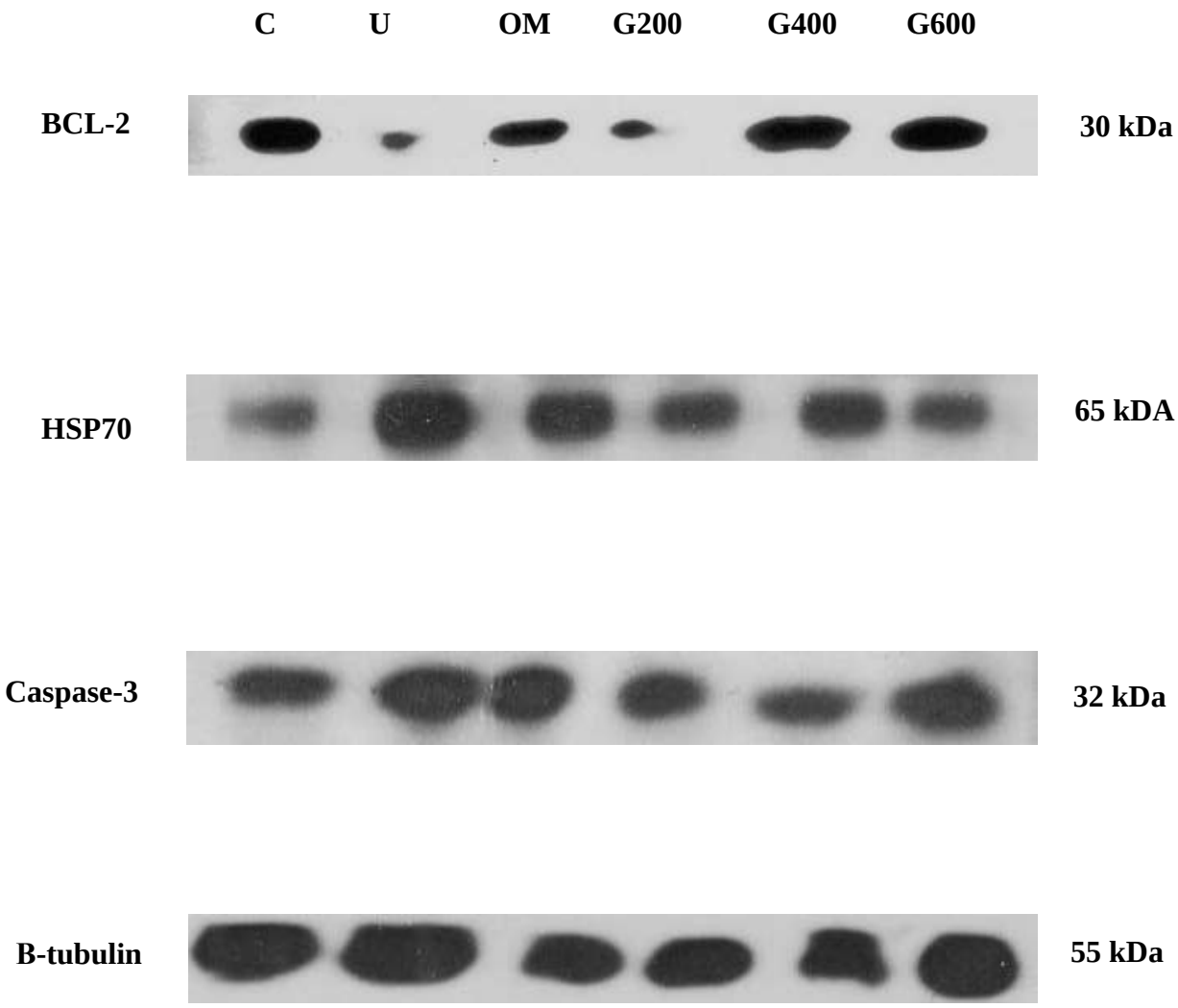


Fig. 4.28 Western blot expression of various cellular regulatory proteins: (A) Normal control group, (B)Ulcer group (C) Omez:(D) ;(E) ;(F) group pretreated with GLME at the dose of 200,400 & 600mg/kg; The GLME has effectively exerted gastro cell protective through maintenance/up regulation BCL2(anti-apoptotic), HSP70(prevent cell from aberrant aggregation and refolding of miss folded proteins), effectively down regulated apoptotic proteins(Caspase-3).

CHAPTER 5

DISCUSSIONS

5.1 Gastroprotective effects of *M. roxburghianus* and *G. lanceifolia* methanol extract: role of phytochemicals

The natural secondary metabolites found in ethno-medicinal plants reported to have antioxidants especially polyphenols, which are performing multiple functions to counteract the free radicals, ROS and modulate the cellular oxidative stress is also nurturing interest due to their redox properties which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelating properties (Pinchuk et al., 2012). *M. roxburghianus* (MR) has long been used in the traditional medicine for their pharmacologically active constituents and therapeutic activities. GC–MS analysis revealed that MR leaves are comprised of bergenin, betulinic acid, β-sitosterol, stigmasterol, and 4-hydroxy benzoic acid as the major phytocompounds (Rana et al., 2005; Roy et al., 2015; Roy et al., 2016). Qualitative and quantitative phytochemical analysis of MR leaves demonstrated that the presence of diverse secondary metabolites and contain significant amounts of total phenols, terpenes flavonoids and alkaloids. Further, LC-MS/MS analysis of MRME showed the presence of 19 phytocompounds wherein bergenin, betulinic acid, gallic acid, hesperidin, tannic acid and myrcetin were found to be the major components identified. Phenols and flavonoids are well recognized for their antioxidant activity and anti-inflammatory capability due to their inhibitory effects on enzymes involved in the production of the free radicals, chemical mediator of inflammation and lipid peroxidation (Farzaei et al., 2015) and these findings are in concordance with our present study results. Antioxidant and anti-inflammatory properties of solvent extracts of MR were confirmed by assessing the different antioxidants and anti-inflammatory models of screening and found that MRME exhibited higher antioxidant and anti-inflammatory potential than the other solvent extracts (Kumari et al., 2017). Lipoxygenase (LOX) and xanthine oxidase(XO) are the enzymes which play a major role in the biosynthesis of leukotrienes which act as proinflammatory molecules mainly responsible for the anti-inflammatory activity and scavenging free

radicals (Nualkaew et al., 2009). Thus antioxidant and anti-inflammatory capacity of MRME can be accredited mainly due presence of its phenolics, terpenes and flavonoids compounds as evidenced by the outcome of this investigation as well as others research findings (Rana et al., 2005; Roy et al., 2015; Roy et al., 2016).

Scientific research on *G. lanceifolia* reported their antibacterial, antidiabetic, antinociceptive, antihyperglycemic, membrane stabilizing activities and antioxidant properties (Bora et al, 2017; Ghosh et al, 2018; Bora et al, 2014). *Garcinia* fruit species possesses numerous bioactive compounds, including xanthones, bioflavonoids, benzoquinones, triterpenes, and benzophenones may be attributed aforesaid biological activities (Bora et al, 2020). The HR-LCMS profile of *G. lanceifolia* was found to be rich source of amino acids, organic acids, fatty acids and flavonoids and bi-flavonoid. The results of this study indicate that *G. lanceifolia* stimulates the production of epithelial cells, which may significantly raise the content of protein in the gastric secretions of the animals that had prior treatment. When it came to ethanol-induced acute hemorrhagic lesions of the stomach mucosa, GLME (400 mg/kg) showed a noteworthy impact. It was shown that *G. lanceifolia* gastric anti-ulcer activity may be related to its capacity to sustain stomach mucus discharge, which results in a decrease in mucosa volume and an increase in surface mucosal area. It was discovered that *G. lanceifolia* exhibited an effective anti-ulcer effect by raising pH and mucus levels while lowering submucosal inflammation and edema. Similarly, several edible and wild fruits have been reported to have anti-ulcer properties (Nesello et al., 2017; Harsha et al, 2017). *Garcinia achachairu*'s isolated xanthones have been shown to have gastroprotective properties thus could be valuable in the control of gastric diseases (Mariano et al., 2016).

Gastric pH and total acidity in the MRME, GLME or omeprazole pretreated rats were at par with normal control group rats while the trend was reversed in the ulcer control group. Ethanol treated ulcer control group produces numerous necrotic lesions and deep ulcers and upsurge the gastric acidity due to the depletion of bicarbonates secretion and mucus production (Al Batran et al., 2013). Lesion counts and ulcer indices, such as pH level, are utilized as indicators of stomach ulcers following exposure to toxins (Adhikary et al., 2011). Reduced hydrogen ion

concentration in gastric juice causes stomach injury because the pH value mainly determines the acidity and volume of gastric discharge (Lullmann et al., 2000). These observations were in line with our results through the observed decrease in gastric acidity and increase in gastric volume and lesion counts. In the ulcerated stomach, the imbalance between the generation of free radicals and scavenging ability may be the cause of the significant decrease in pH and rise in gastric volume (Fahmi et al. 2019 a, b). It was found that in rats with stomach ulcers, the protective and therapeutic effects of mallotus extract were successful in raising pH levels and reducing the number of lesions and gastric acid flows.

Pre-treatment with MRME and GLME400 significantly increases the GWM content in the gastric glands similarly *Strobianthes crispus* leaf extract enhanced the GWM in the ethanol induced ulcer in the rats (Mahmood et al., 2011). The increase in Alcian blue staining and a characteristic enhance in the intensity of carmine PAS histochemical staining of the gastric tissues that release mucopolysaccharides in the MRME and GLME400 pretreated group's exhibits the gastroprotective effect of MRME. The PAS staining technique used to detect mucopolysaccharides in in stomach tisses, including gastric mucus. Increased mucus in the stomach is directly propotionsl to level of PAS stain in the tissue. According to Halabi et al. (2014), ulcer-control rats treated showed the lowest PAS stain intensity. The present study found that ethanol decreased mucus barriers, while MRME and GLME treatments significantly improved mucus layer thickness of the stomach mucosa, as seen by higher PAS expression (fig 4.5 & 4.22). Stomach mucus may play an essential role in epithelial defence in MRME & GLME pretreated group. The results revealed that MRME/GLME guards the mucosa by concealing excess mucus in response to significant gastrointestinal damage. Flattening of the mucosal folds in the gastric tissue has been associated with the progress and prevention of gastric lesions. The MRME pretreatment (200 mg/kg) and GLME (400 mg/kg) restored the flattening of the mucosal folds in the GW upon treatment with ethanol (Fig. 4.1 I). Thus, possible mechanisms by which the GM is protected by MRME involves the flattening of mucosal folds that: (i) shielded the GM by increasing the mucosal surface area exposed to necrotizing agents and further minimize the quantity of the gastric

irritants that come into contact with the rugal crest (Al Batran et al., 2013) and (ii) reduced the gastric motility which inhibit the formation and advancement of necrotic gastric lesions (Hajrezaie et al., 2015).

Plant extracts can be portrayed as a new perception for the management of gastric disorders because the biochemical constituents present in these milieus can perform as a phytocomplex persuading the lessening of the effects of the offensive agents and strengthening of the defensive factors (Kwiecien et al., 2014).

5.2 Acute tocixity and sub-acute of MRME and GLME:

No reports of its toxicity (MRME and GLME) evaluation have been reported to until now. The purpose of acute toxicity testing is to ascertain how a single dose affects particular test animal species. Toxicological investigations are essential to determine safety, highlighting the importance of evaluating the toxicological profile before selecting a safe dose (Büttler et al., 2023). This allows for the determination of the plant's inherent toxicity as well as the consequences of an acute overdose (Palmer et al., 2019). The rat has been one of the most common mammalian species used in preclinical studies ranging from pharmacology to safety assessment. The use of rats as safety models is now mandated by international recommendations for both chemicals and pharmaceuticals (Hedrich and Bullock, 2004). Rats' ease of maintenance lowers research costs, and their accelerated lifetime allows all life stages to be investigated (Hsuetal., 2011).

The oral administration of MRME up to 3 g/kg is safe and caused no toxicity and no mortality in the acute toxicity trial. Medicinal plant extracts and their constituents are mostly regarded as safe to human consumption. *Morinda lucida* leaf extract was shown to be non-lethal at 6400 mg/kg body weight based on the acute oral toxicity and LD50 results. The findings for the parameters measuring liver and kidney function showed that eating leaf extract from *Morinda lucida* had no harmful effects on these systems (Adeyemi et al., 2023). In rodents, *Salvia przewalskii* Maxim (SPM) extract was found to have low or no toxic effects on both acute and subacute toxicities (Li et al., 2010). Even at higher dosages than those deemed therapeutic, the crude extract (CE) from rhizomes of *Limonium brasiliense* seemed to be safe,

according to evaluation conducted 90 days after delivery in rat model (Antonelli-Ushirobira et al., 2015).

The oral acute and subacute toxicity of *Garcinia lanceifolia* was first explored. All three doses (1000,2000, and 3000mg/kg) of acute toxicity test resulted 100% of death in all experiment groups in the experiment period, implying that the extract had toxic effects. Rats that died in an acute toxicity investigation exhibited hypoactivity and oral haemorrhage (clinical sign). These findings were consistent with reports of accidental natural eucalyptus oil intoxications (Webb and Piit, 1993; Darban et al., 1998). Though there is no death recorded in rats with administration of 3000 mg/kg of stem bark aqueous extract of *Musanga cecropioides.* However, among the behavioral indicators of extract that have been noticed are anorexia, tachypnea, restlessness, irritability, bilateral eyelid narrowing, and aberrant posture, which was typified by the head being pulled between the hind limbs (Adeneye et al., 2006). There have been rare reports of poisonings in sheep caused by the intake of galega herbs (Puyt et al., 1981). Microscopic analysis revealed alveolar hemorrhage and sinusoidal congestion in the liver administrered with 5g/kg body weight of *Galega officinalis* herb in rats (Rasekh et al., 2008). 6.3 g/kg body weight of dried plant material of *Verbesina encelioides* caused significant glomerulonephrosis and liver congestion, along with cellular degradation and lipid alterations in the sheep (Lopez et al., 1996). If the extract is lethal in the body, it has a direct effect on its components, including red blood cells, white blood cells, platelets, and hemoglobin. These components' ranges are either drastically decreased or increased from normal. It shows that the extract's toxicity may affect both the body's immune system and organ function (Osano et al., 2016).

Sub-acute toxicity was assessed over 28 days at dosages of 50, 100, and 200 mg/kg/day All three doses (50,100, and 200mg/kg) of GLME resulted in no significant changes in weight gain or food consumption/water intake during the experiment period, implying that the extract had no negative effects. There were no significant differences in rat physiological or metabolic activity as compared to the control. At the end of the subacute period, the organs were extracted and weighed.

There were no significant differences in the relative organ weight of the spleen, liver, and kidney between the control and GLME (50, 100, and 200mg/kg) treated groups. Histopathological characteristics are important to assess the toxicity of plant extracts and its impact on organs (Traesel et al., 2014). Our histology studies revealed that the liver, spleen, and kidneys were the primary targets of GLME acute and sub-acute toxicity. The pathological findings showed that 1000,2000, and 3000 mg/kg/day doses of GLME had serious damages on liver, spleen, and kidney with main lesions of granular and vacuolar degeneration and vascular congestion, The histological analysis revealed kidney injury in rats treated with 1000,2000, and 3000mg/kg of GLME, such as vessel congestion, shrunken glomeruli, vacuolar degeneration and renal tubules, which may result in impairment of renal tubular reabsorption and glomerular filtration, leading to inability to absorb various ions and severe tissue edema. These findings revealed that high doses of the GLME could harm rats' organs, including liver damage, glomerular shrinkage, and partial spleen cell death. All lesions can be validated by histological sections. However, the particular mechanism of harmful activity requires additional investigationwhile 50,100, and 200 mg/kg/day doses of GLME of subacute toxicity had no damages on liver and kidney, while acute GLME intoxication caused pathological and function damages in the liver and kidney of rats.

5.3 Histochemical analysis and antioxidant systems involved in gastroprotection in MRME & GLME pretreatment

In the present study,histopathological analysis (H&E, PAS and immunochemical staining) revealed that ethanol treated rats severely induced injury to the mucosa, submucosa and muscularis regions of gastric tissues, disruption of the vascular endothelium, edema and leucocyte infiltration of the submucosal layer(fig.4.4(I)) whereas consistent reduction in histological anomalies, inhibition of edema and leucocyte infiltration and protection of gastric tissue from ulcers were observed in MRME pretreated groups as reported by Saeed AL-Wajeeh et al., (2016). In animal models, ethanol is often used to cause stomach lesions (Abd-Alla et al. 2016; Fahmi et al. 2019). It is characterised by apoptosis, oxidative stress, inflammation, and the production of free radicals (Fahmi et al. 2019; Choi et al. 2009). In this study, the rat

ulcer model induced by ethanol (oral administration, 5 mL/kg) was used for screening a new gastroprotective phyto-therapeutic agent (MRME) and GLME400 because ethanol damages the gastric tissue by diverse courses as excessive generation of free radicals and ROS, depletion of mucus, obstruct the protective factors of GM membrane, the reduction of antioxidant defense enzymes and inhibition of prostaglandin synthesis (Liu et al., 2021). The potential of ethanol to encourage leukocyte infiltration at the ulcer site may also be related to its ulcerative mechanism (Figueiredo et al., 2022). According to Ottman et al. (2022), neutrophils are specific immune cells that are thought to be crucial players in the cellular defense system because they initiate phagocytosis and produce reactive oxygen species (ROS).

The mucosal and submucosal layers of the stomach will develop lesions as a result of neutrophil activation and infiltration. Consequently, one of the key ways that efficient antiulcer medicines/extract work is by decreasing the infiltration of inflammatory cells (Beiranvand, 2022). As displayed in the results of the histological analysis(fig.4.4-(I)), the ulcer control group's stomach histological examination results showed that there were significant hemorrhagic stomach injuries along with enhanced leucocyte (neutrophil) penetration, which resulted in submucosal coating edema. MRME(200mg/kg) & GLME (400mg/kg)-fed experimental animal groups, demonstrated gastroprotective effects right before the the administration of the absolute ethanol.

In addition, the major phytochemical constituents of MRME are polyphenols (bergenin) and terpenes that appears to be a crucial factor in gastroprotective activity by subsiding the severity of ulcers, increasing the amount of GWM, decreasing the membrane permeability, recruiting of anti-inflammatory mediators and inhibition on prostaglandin stimulation, production of mucus content and their antioxidative effect (Abe et al., 1980; Farzaei et al., 2015; Kwiecien et al., 2014; Patel et al., 2012). Anti-ulcer activity was reported other Garcinia spp. *Garcinia cambogia'*s fruit extract potential as an antiulcerogenic agent was validated by its ability to reduce acidity and boost mucosal defense in the stomach regions (Mahendran et al., 2002).

The capacity to boost the gastric mucosa's protective components accounts for the anti-ulcer action of *Garcinia achachairu* (Mariano et al., 2016)

Gastric immune cells alterations may be implicated in the pathophysiology of GU. Since cytokines, chemokines, and neuroactive chemicals released from immune cells may affect gastrointestinal motility and sensitivity. Eosinophils and mast cells are taking part in the preservation of the homeostasis of the gastrointestinal epithelium, tissue remodeling processes, help macrophages with the phagocytosis processes and immunomodulatory actions (Lee et al., 2013). In line with these results, the activation and infiltration of eosinophils and mast cells appear to be involved in the initial processes of inflammation that form these lesions in the ethanol treated group. But, pretreated MRME groups prior to ethanol administration significantly reduced infiltration of mast cells number as well as eosinophil mobilization into submucosa and mucosa tissue assisted to prevent GUs in rats (Mutmainah et al., 2014). Ethanol easily permeates the stomach mucosa because it dissolves the protecting mucus and exposes the mucosa to the hydrolytic and proteolytic actions of pepsin and hydrochloric acid. It also causes significant microvascular alterations, including strong vasoconstriction and arteriolar dilatation, which result in mucosal capillary engorgement. The pathophysiology of stomach mucosal injury is linked to decreased secretion of bicarbonates and the production of ROS, which seems to be necessary for the production of lipid peroxides, as well as decreased cell anti-oxidative enzyme activity (Ahmed et al., 2022). There is also evidence that instigation of GUs and its pathogenesis are mediated mainly by cellular oxidative stress via generation of free radicals, ROS and lipid peroxidation that causes malfunction of membrane fluidity, ion transport, membrane integrity and cellular activity (Bandyopadhyay et al., 2001). Antioxidant enzymes participate as a key defensive component in protecting the GW against a range of necrotic agents (Bhattacharyya et al., 2014). MRME and GLME pretreated rats demonstrated significant antioxidant activity with decreased levels of MDA and elevated levels of SOD and GST in response to oxidative stress induced by ethanol. Antioxidants are liable to protect the gastric tissues from ulcer formation by means of radical scavenging activity (Kwiecien et al., 2014). Similarly, *Garcinia indica* fruit rind aqueous extract doses (400 mg/kg and 800 mg/kg) (GIE) exhibited

significant anti-ulcer activities against ethanol induced rats via attenuating the ulcer elevated levels of MDA and restored the ulcer-depleted levels of antioxidant enzymes GSH, SOD, CAT, GPx and GR(Panda and Khambat, 2014). Pre-treament of methanolic extract of folklore medicine *Garcinia kola* prevented the gastrointestinal damage caused by ethanol by decreased the ulcer score, morphological damage score, gastric wall thickness, and lipid peroxidation ($p<0.05$) and enhanced the gastric mucosa's structural integrity attributed to it flavonoid constituents (Ige et al., 2012). The potential relationship between kolaviron a biflavonoid isolated from *Garcinia kola* was linked to its antioxidant qualities (restoration of CAT, SOD and GSH activities in gastric mucosa) thus have gastroprotective action (Olaleye and Farombi, 2006). *Garcinia cowa* fruit extract rich in phenolic, flavonoid α-mangostin and xanthochymol content showed marked anti-ulcer activity in ethanol induced rats by inhibition of lipid peroxidation and restoration of adherent gastric mucus, NP-SH content, and histological architecture (Gupta et al., 2021).

According to previous research, ROS play a role in the pathology of gastric mucosal damage caused by ethanol (Cemek et al., 2010; Liju et al., 2015; Sidahmed et al., 2015). ROS are unpaired molecules that are produced naturally by peroxisomes and mitochondrial respiration, and they catalyze various redox processes in living things (Uner et al. 2010; Caksen et al. 2014). According to Aslan et al. (2012), oxidative stress arises when the production of ROS accumulates excessively and surpasses the capacity of the cellular antioxidant system. The ROS reaction against the cell membrane causes lipid peroxidation, which produces high lipid peroxidation products including MDA and oxidative gastrointestinal injury. Lipid peroxidation is measured with MDA. Gastric ulcers have been caused by elevated MDA levels, and MDA synthesis has been observed to rise after ethanol-induced injury to stomach tissue (Birdane et al. 2007; Cemek et al. 2010).

Macuna pruriens has been reported to enhance antioxidant production and lower MDA levels in the respective pre-treated groups in an experimental investigation of its gastroprotective action (Liju et al. 2015). Among all GLME dosages used in this investigation, 200 and 400 mg/kg markedly reduced MDA levels ($p<0.001$). This

result highlights GLME's potential to increase antioxidant levels, scavenge free radicals, and reduce lipid peroxidation.

GSH is a tripeptide that is an important non-enzymatic cytosolic antioxidant that serves as a reductant and cofactor for specific antioxidant enzymes (Cemek et al., 2006; Ertug et al., 2013).

According to a previous study, zerumbonein may enhance the protective effects of gastric mucosal factors, especially when there is a notable rise in the endogenous antioxidant GSH and a corresponding decrease in the amount of lipid peroxidation (Shui and Peng 2004). According to our research, the GSH content increased after administration of GLME 400 mg/kg. Omeprazole is a drug used to treat stomach ulcers. In our investigation, GSH levels significantly increased with GLME 400 mg/kg treatments, which were comparable to omeprazole (20 mg/kg) (Fig 4.23A). Our findings demonstrated that 400 mg/kg of GLME has a greater gastroprotective effect.

NO free radical is generated by phagocytes and endothelial cells, reacts with superoxide radical, forming peroxynitrite anion ($ONOO^-$) and plays an important role in inflammatory process causing vascular collapse associated with ulcerative colitis (Saijo et al., 2010). In this study, the level of NO radical was significantly reduced in a dose dependent manner by MRME and GLME, explaining its role in treatment of inflammation and for wound healing (Gangwar et al., 2014).

5.4 COX-I and COX-II protein modulation

COX primarily responsible for prostaglandin synthesis is composed of two isoforms: a constitutive COX-I is expressed in the gastrointestinal epithelium, endothelial cells, and platelets while an inducible COX II under pathophysiological conditions like inflammation in inflammatory cells and fibroblasts in the stomach mucosa. COX II is induced in inflammation by various stimuli—such as cytokines, endotoxins, and growth, gives rise to prostaglandins (Mitchell et al., 1995). Prostaglandin (PGE2) synthesis in the gastrointestinal (GI) tract is mostly carried out by COX I, whereas prostaglandin production from the transformation of arachidonic acid at the site of inflammation is conducted by COX II (Morteau, 2000). In both humans and rat

(animals), reduced prostaglandin levels at the stomach mucosa are known to aggravate pre-existing gastric ulcers (Poonam et al., 2005). COX isoenzymes are blocked by NSAIDs. It is well recognized that prostaglandins are essential for safeguarding the stomach mucosa by promoting the production of mucus, bicarbonate secretions, and mucosal blood flow and stimulate angiogenesis (Yandrapu and Sarosiek, 2015).

In the present study constitutive adequate level of COX I was observed in the stomach mucosa of control rats while an inducible high level of COX II was evident in the ulcerated ethanol induced rat groups. COX-1 expression in gastric ulcer tissues in ethanol treated rats showed lower expression of COX-1 in gastric stomach tissues, which was considerably increased by MRME pre-treated rat groups. Reduced PGE2 synthesis due to COX-1 inhibition has a significant role in the pathophysiology of gastric ulcers (Poonam et al., 2005). Therefore, the decreased cyclooxygenase expression in epithelial could contributes to poor ulcer healing. *H. pylori* infection was associated with significant increase in vivo PGE2 synthesis. This was associated with increased immunoreactivity for both COX I and COX II in the epithelial cells of profilerative zone and COX II in the inflammatory cells of the lamina propria (Shen et al., 2006). However, some studies have demonstrated that COX-2 inhibition/downregulation showed beneficial effects on gastric ulcers (Sánchez Fidalgo et al., 2004; Aziz et al., 2019). Even prostaglandin production was readily suppressed by a COX-I inhibitors (Ribeiro et al., 2004). In lipopolysaccharide-treated Caco-2 cell lines, natural ginger extract reduced COX 2/PGE2 overproduction, thereby suppressing inflammatory responses (Shin et al., 2020). COX II protein was found be induced in macrophages and fibroblast in gastric ulcer and *H. pylori* related gastritis suggesting its role in the gastric tissue repair process (Gudis and Sakamoto,2005). MRME pretreated rat groups exhibited modulation of COX I enzymes and down regulation of COX II at 200 mg/kg dose. Similar to present study *Cissus quadrangularis*, *Plumbago zeylanica*, *Terminalia bellarica* and *Terminalia chebula* were found to inhibit COX-2 activity as compared to COX-1. The extracts of *T. bellarica* (73.34%) and *T. chebula* (74.81%) showed significant COX-2 selective inhibition in the anti-inflammatory activity (Shaikh et al., 2016).

Oxyresveratrol, a bioactive phytochemical ameliorates ethanol induced gastric ulcer via down regulation COX-2(Aziz et al., 2019). These results indicate that MRME inhibited pro-inflammatory cytokines including TNF-α, which in response inhibited the inflammatory response in the stomach tissue. Also, it decreased COX-2 expression. (Kwon et al., 2012; Margraf et al., 2019) There is considerable suppression in the expression levels of COX-2 isoenzyme, which may be one of the reasons for the anti-inflammatory action of MRME.

5.5 BCL2 and BAX protein modulation:

A well-established cellular biological event is reactive oxygen species (ROS) induced signaling pathway mediated activation of programmed cell death (Panieri et al., 2013). Numerous intracellular and extracellular signaling molecules control cellular apoptosis. A key mechanism regulating apoptosis is changes in the ratio of the pro-apoptotic protein Bax to the anti-apoptotic protein Bcl-2(Fan et al., 2014). It is known that ethanol causes apoptosis via interfering with the mitochondrial system mechanisms that controls the expression of the anti-apoptotic Bcl-2 function primarily by blocking the apoptosis pathway and pro-apoptotic Bax proteins regulation trigger the process of apoptosis (Ola et al., 2011). The Bcl-2 gene product is a negative regulator of apoptosis that counteracts the effects of pro-apoptosis by forming a hetero-dimer complex with Bax (Pinto Rodrigues, 2012). ROS was found to mediate via the lowered the expression i.e. down regulation of anti-apoptotic Bcl-2 and overexpression (up regulation) of Bax pro-apoptotic proteins (Mayola et al.2011). Several scientific studies have demonstrated the link between apoptosis and the emergence of gastric ulcers (Konturek et al., 1999; Houghton et al., 1999). The ethanol-induced gastric ulcer was associated with increased Bax protein expression, decreased Bcl-2 protein expression, and elevated Bax/Bcl-2 ratio (El-Din et al., 2021). Excessive induction of apoptosis over time will eventually weaken the stomach mucosa, causing the mucosa to malfunction and ultimately lead to gastric ulcers (Konturek et al., 1999). In the present study it was revealed that, at a molecular level, the ingestion of ethanol led to a noteworthy down regulation of the anti-apoptotic protein Bcl-2, facilitated by ethanol induced inflammation and reactive oxygen species. The restoration of Bcl-2levels and the regulation of pro apoptotic

Bax expressions demonstrated unequivocally that the stomach mucosa's anti-apoptotic capacity had been boosted in the MRME200 and GLME400 pretreated groups compared to the ethanol induced ulcerated groups. Thus, *M. roxburghianus* and *G. lanceifolia* possess anti-apoptotic qualities and may be able to lessen the gastric damage induced by ethanol. Similarly, to present study *Hyphaene thebaica* Mart. extract mitigates oxidative stress and Bax- and Bcl-2-mediated apoptosis in ethanol-induced gastric ulcers in rats (Taha et al., 2022). *Ligularia fischeri* root extracts found to be cytoprotective against ulcerative colitis in mice through activation of Bcl-2/Bax signaling (Fu et al., 2022). QRZSLXF, a Chinese medicinal herb recipe found to be effective against ulcerative colitis via the DOR-βarrestin1-Bcl-2 signal transduction pathway (Fan et al., 2014). When *Cupressus sempervirens* leaf ethanolic extract was administered to rats exposed to indomethacine, it enhanced the expression of p53 protein and lowered the expression of apoptotic Bcl-2 protein (Koriem et al., 2014).

5.6 HSP70 expression

HSP70 proteins are endogenous cytoprotective factor to protect the protein structure, stability and folding pattern from cellular toxicity or physiological injury through repair or removal of damaged proteins via refolding process (Tóth et al., 2015). In the current investigation Ethanol pre-treated ulcer control group there was inhibition of the expression of HSP70, as absolute ethanol enhanced the generation of reactive oxygen species (ROS), which increased the presence of pro-apoptotic proteins and inhibited the development of HSP 70 protein. Oxidative stress degrades DNA, proteins, and lipids; it leads to lipid peroxidation, cellular death, and tissue damage (Recknagel et al.,2020). Stomach epithelia were protected in experimental groups administered MRME/GLME due to up-regulation of HSP 70. According to various researchers, the up-regulation of HSP 70 is linked to stomach defense against absolute ethanol, possibly through the reduction of ROS-mediated stomach oxidative pressure (Tanak & Mizushima, 2009; Shareef et al., 2022; Balaky, 2024).

Pretreatment with ethyl acetate extract of *Annona muricata* L. leaves triggered the overexpression of HSP70 and the downregulation of BAX proteins gastroprotective

effect in ethanol induced injury in rats as shown by immunohistochemical labeling (Moghadamtousi et al., 2014). Phenolic phyto-compound gallic acid exhibited gastroprotective effect in against ethanol-induced gastric ulcerogenesis via immunomodulation of HSP70 and BCL-2-associated X proteins (Abdelwahab, 2013). The expression of the BAX protein is down regulated and the HSP70 protein was upregulated was recorded as the gastroprotective factors in the stomach epithelium pretreated with *Papaver decaisnei* against ethanol-induced gastric ulcers in rats (Jabbar et al., 2022). Key component of *Paeonia lactiflora* Pall, paeoniflorin, has the ability to induce heat shock proteins (HSPs) in cultured mammalian cells without any apparent toxicity (Asai et al., 2011). Gastric acid secretion, heat-shock protein 70 (HSP70) and pro-apoptotic protein (BAX) modulation, cytoprotective barriers, and antioxidant capacity are essential defensive mechanisms are found to be key mechanism in gastroprotection by medicinal plants of Malaysian region (Wasman et al., 2022). The present study results are in line with those of other investigations as mentioned above. HSP70 appears at sites of cellular stress and protects against harm. In stomach ulcers, HSP 70 protection involves stimulating normal protein generation by eliminating injured proteins (Mosser et al., 1997).

5.7 Caspase-3 expression

It is generally known that caspases play a crucial role in the process of apoptotic DNA fragmentation (Salvesen and Dixit, 1997; Majtnerová and Roušar, 2018). Caspase-3 is an apoptotic cysteine protease is activated in a series of processes linked to programmed cell death, or apoptosis (Fan et al., 2005). Cell death resulted from the activation of apoptotic BAX protein translocation to the mitochondria, which releases more pro-apoptotic proteins into the cytoplasm, promotes the creation of apoptosomes, and ultimately activates Caspase3, the apoptosis executor (Solano-Gálvez et al., 2018; Saadaoui et al., 2020). Research has demonstrated the involvement of Bax and Caspase-3 in mitochondrial damage-induced apoptosis and the subsequent impairment of stomach mucosal integrity following ethanol induction also found in the present study (He et al., 2019). The caspase-3/E-cadherin pathway contributes to the *H. pylori-induced* death of gastric epithelial cells (Yang et al., 2017). An increase in mucosal expression of caspase-3 activity and widespread

numerous hemorrhagic lesions and significantly increased gastric epithelial cell death were formed by indomethacin induction in rat model is conformity with present study by ethanol induction (Piotrowski et al., 1999). Pretreatment with MRME and GLME exerted down regulation of caspase-3 in ethanol induced rats similarly gallic acid (3,4,5-trihydroxybenzoic acid, GA) is a phenolic acid occur in many medicinal pretreatments clearly reduced the immunohistochemistry expression of Bax and Caspase-3 in ethanol induced rats (Zhou et al., 2020). Salusin-α and salusin-β are bioactive peptides administered rat groups, significantly reduced mucosal injury and caspase-3 expressions compared control in ethanol induced rat model (Tanyeli et al., 2017). Ursolic acid (UA) isolated from the dichloromethane/methanol extract of shoots of *Ochrosia elliptica* Labill. significantly down regulated the Caspase-3 activity in ethanol induced gastric rat model (Elshamy et al., 2020).

5.8 TNF alpha expression:

Tumor necrosis factor alpha (TNF alpha) is an inflammatory cytokine having pleoitropic functions produced during acute inflammation and is secreted by macrophages and monocytes. It triggers a variety of signaling events in cells that result in necrosis or apoptosis (Parameswaran and Patial, 2010;). In gastric epithelial cells, external factors such as ethanol, NSAIDs, indomethacin has ability to activates NF-κB, which is then translocated to the nucleus and increases the production of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 (Sinha et al., 2015). Even TNF-α alters the gastric mucosa's apoptotic cell death, which is brought on via the caspase-3 pathway. Thus, TNF-α is a pro-inflammatory and also pro-destructive factor for gastric tissue because it recruits neutrophils and other leucocytes by inducing adhesion molecules in both types of cells. Supplementary ability of pro-inflammatory cytokines is the generation of superoxide molecules by neutrophil buildup and activation, which ultimately causes disruption of the microcirculation, suppression of angiogenesis at the ulcer margin, the generation of free radicals, and thus delays ulcer healing. The reduction in stomach ulcers in the rats is directly correlated with the modification of these cytokines. The potential of PGE2 to impede TNF-α synthesis could account for the decline in TNF-α level. Therefore, it could be assumed that lowering the TNF-α level would speed up the healing of ulcers. TNF-α

and excessive exposure to oxidative stress on the stomach mucosa have been shown to promote gastric epithelial apoptosis.

In this respect, our study looked at TNF-α expression levels, and the findings showed that the ulcer control group had significant higher levels, but the groups that received MRME had significantly attenuated TNF-α expression levels. In the present study MRME pretreated rat groups exerted significant down regulation of TNF-α cytokine level is conformity with below scientific studies. Cavidine an alkaloid from *Corydalis impatiens* effectively suppressed the up-regulation of cyclo-oxygenase-2 (COX-2) expression and the activation of the nuclear factor-kappa B (NF-κB) pathway, as well as the levels of interleukin-6 (IL-6) and TNF-α (Li et al., 2016). Rats treated with ethanol-induced gastric ulcers are less affected by *Allanblackia floribunda* seed extract because it inhibits TNF-α and INF-γ levels (Armah et al., 2021). The antiulcer properties of *Phyllantus. reticulatus* was attributed to the down regulation of IL-8 and TNF-α mRNA expression levels (Izhar et al.,2021). *Camellia japonica* significantly down regulated the gene expression of inflammatory cytokines and enzymes, including TNF-α, IL-6, IL-1β, iNOS, and COX-2(Akanda and Park, 2017). Garcinol, a polyisoprenylated benzophenone derivative reported from *Garcinia indica* exhibited anti-ulcer mainly attributed to decrease the expression of DNA polymerization markers (mTOR, cyclin D1), cell proliferation markers (PCNA), inflammatory markers (COX2, TNF-α, and IL-1β) associated with elevation of d anti-inflammatory markers (IL-4 and IL-10) (Alatawi et al., 2023). Our findings are consistent with a prior work by Du et al., 2013 that found that *Veronicastrum axillare* treatment improved gastric ulcers, and that the lower increased TNF-α levels were the cause of the anti-ulcer activity.

Consuming ethanol may activate the innate immune system, which could result in the release of pro-inflammatory cytokines like TNF-α and IL-6 (Montesinos et al., 2016). One possible explanation for the observed rise in TNF-a production in stomach ulcerated tissue is the necrotizing effects of ethanol. TNF-α and other pro-inflammatory cytokines are known to trigger apoptotic cell death by triggering the

caspase family of proteases. Nuclear factor kappa B and other cytokines are synthesized as a result of TNF-α overlapping and synergistic activities (Zhou et al., 2006). Elevated TNF-α and IL-6 activates neutrophils, lymphocytes, and monocytes/macrophages at the site of inflammation, which in turn triggers a variety of oxidative bursts, toxic metabolites, and lysosomal enzymes that lead to local tissue death in peptic ulcer disease (Bhattacharyya et al., 2014). TNF- α levels in the stomach mucosa relate with the degree of inflammation. Moreover, stomach ulcer healing benefited from the suppression of inflammatory mediators. In the present findings of this investigation revealed that MRME has the ability to reduce the level of pro-inflammatory cytokine mediator (TNF- α) (Fig. 4.14), demonstrating its anti-inflammatory impact in ethanol-induced gastric ulcer. In the present study, we found that MRME's anti-inflammatory impact may be linked to down regulation of TNF alpha activity in rats with ethanol-induced stomach ulcers. Immunohistochemistry and Western blot tests revealed that protein expression of TNF alpha increased significantly in rats with ethanol-induced gastric ulcers and significantly decreased in groups pre-treated with MRME.

5.9 PCNA expression:

PCNA is one of crucial protein play a vital role in transcription, chromatin structure, DNA replication, and repair. Its main function is to regulate many other proteins that are involved in the synthesis, repair, and recombination of DNA. In the present study there was a notable upsurge in PCNA immunoblot-expression in the MRME (200mg/kg) group compared to ulcerated group & among all group of *M. roxburghianus*. This conclusion aligned with the findings of El-ghazouly and Yassien,2024 who hypothesized that an increase in PCNA could be a marker of stem cells multiplying and differentiating to promote damaged cell regeneration and, ultimately, helps in mucosal healing. According to some studies, increased PCNA immune expression is an indication that DNA repair is taking place; in non-proliferating cells repairing DNA, it can replace abnormal nucleotides released after injury (Mousa and Fahmy, 2023). The integrity of the stomach mucosa depends on maintaining a balance between cell growth and death, cell proliferation is crucial for gastric mucosal reconstruction (Oncel and Basson, 2022). The healing of stomach

ulcers is significantly influenced by cell proliferation. Undifferentiated epithelial cells proliferate and migrate to cover the ulcer's base, causing mucosal cell regeneration. Proliferation and apoptosis must be balanced for epithelium to stay maintained. According to Negroni et al., (2015), the absence of a continuous epithelial barrier would expose the mucosa to infection and chemical damage, which could delay the healing of ulcers.

According to Vasconcelos et al., (2010), PCNA is a nuclear peptide that is produced during cell division. The current findings showed that the MRME-treated group enhanced PCNA expression, but ethanol reduced mucosal PCNA expression. According to Sinha et al. (2012), Moringa oleifera ethanolic extract may have an anti-ulcer impact via preventing apoptosis in addition to causing increased mucosal proliferation for these reasons.

5.10 DNA protecting property of MRME assessed by tunnel assay

Localization of apoptotic cells that have experienced significant DNA degradation in the last stages of their life cycle is the aim of the TUNEL assay (Collins et al., 1997). Fragmentation of nuclear DNA by nuclease is one of the hallmarks of apoptosis. The protein family that carries out the procedure of cell death, caspase, activates DNA fragmenting enzymes (Majtnerová and Roušar, 2018). Gastric ulcer formation is determined by the equilibrium between apoptosis and the proliferation of cells. As ethanol consumption results in destruction of the stomach mucosa, apoptosis plays an important role (Zhou et al., 2020). By using the TUNEL technique, we were able to confirm earlier findings by observing that apoptosis was present in the stomach tissues and that the ulcer model group’s gastric mucosa displayed elevated apoptosis (Chen et al., 2016). In this study, the TUNEL assay was utilized to identify DNA fragmentation that causes apoptosis, and immunohistochemistry markers for caspase-3 and Bcl2 were used to determine the role of apoptosis in the group of ulcers caused by ethanol. The TUNEL assay finds DNA fragments that cause cell death. Evidence of apoptosis is provided by the TUNEL assay, upregulated caspase-3, and downregulated Bcl2, which are recognized indicators of apoptosis (Farhadi et al., 2015). In the present study, the ethanol-treated group demonstrated a significant

increase in TUNEL-positive nuclei, and caspase-3 expression and a decrease in Bcl2 expression relative to the control group verified the occurrence of apoptosis in the ethanol-treated group. Pre-treatment with MRME in a dose-dependent manner resulted in a significant decrease in TUNEL positive nuclei and caspase-3 activity as well as an increase in Bcl2 marker expression, suggesting that MRME may have an anti-apoptotic activity effect. Thus, the TUNEL assay was can be used to examine the anti-apoptotic effect of MRME. The results showed that the administration of ethanol significantly increased the number of TUNEL-positive cells. However, the number of apoptotic cells in the stomach tissue of rats who had MRME pre-treatment significantly decreased (($P < 0.001$; Fig. 4.16). These results show that MRME's potential to prevent apoptosis could help protect rats against ethanol-induced stomach ulcers.

CHAPTER 6

CONCLUSIONS

MRME leaves are the rich source of phenolics terpene flavonoid and alkaloid compounds such as bergenin, betulinic acid, gallic acid, hesperidin, tannic acid, myrcetin, luteolin, kaempferol, quercetin, protocatechuic acid, hesperitin, gentisic acid, naringenin, ellagic acid, chlorogenic acid, rutin, genistein, chrysin and coumarin exhibiting strong antioxidant, with high reducing power capacity, maximum anti-lipid peroxidation and anti-inflammatory properties. The MRME even at higher doses of 1000 mg/kg and 3000 mg/kg were found to be safe. MRME exerted strong dose dependent gastroprotective activity against ethanol induced gastric injury/ulcer via amelioration gastric juice pH, acidity, and strengthening of gastric wall mucus. MRME found to downregulate lipid peroxidation (MDA), and restored the antioxidant markers such as SOD and GST and NO in ethanol induced MRME pretreated rats. The MRME has effectively exerted gastro cell protective through maintenance/up regulation following regulatory gastric proteins such as COX-I, COX-II (responsible for PGE & NO synthesis, gastric flow), BCL2(anti-apoptotic), PCNA (DNA replication & cell cycle regulation marker) and HSP70(prevent cell from ab errant aggregation, and refolding of miss folded proteins), effectively down regulated apoptotic proteins (BAX and Caspase-3). Tunnel assay validated DNA protective property of MRME.

GLME fruit rinds are the again rich source of phytochemicals such as amino acids (5-Aminolevulinic acid, L-Glutamic acid, L-Dopa, Leucine, L-Cystine, L-Serine, L-Glutamic acid and 3-Hydroxyanthranilic acid), organic acids (Pyrrole-2-carboxylic acid, L-(-)-Malic acid and Citric acid), fatty acids and their derivatives (Hexadecanamide, oleamide and 16-Hydroxyhexadecanoic acid), flavonoids (Vitexin, (1S)-1,5-Anhydro-2-O-(6-deoxy-α-L-mannopyranosyl)-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-6-yl]-D-glucitol, Scutellarin, Luteolin) and courmarin (4-Hydroxycoumarin). Acute toxicity assay and histopathogical observation of organs such liver, kidney and spleen recorded for higher doses of GLME (1000 mg/kg, 2000 mg/kg and 3000 mg/kg) found to be toxic. Sub-acute

toxicity assay found that lower doses (50 mg/kg, 100 mg/kg and 200 mg/kg) found to be safe. Gastroprotective effect of GLME does not followed dose dependent activity, compared high dose (600 mg/kg) lower dose 400 mg/kg was found more effective. Similar to MRME, GLME also exerted gastroprotective activity against ethanol induced gastric injury/ulcer via amelioration gastric juice pH, acidity, and strengthening of gastric wall mucus. GLME found to down regulate lipid peroxidation (MDA), and restored the antioxidant markers such as SOD and GST and NO in ethanol induced GLME pretreated rats with maximum protection was recorded at 400 mg/kg dose. GLME at the dose of 400 mg/kg; The GLME has effectively exerted gastro cell protective through maintenance/up regulation BCL2 (anti-apoptotic), HSP70 (prevent cell from aberrant aggregation and refolding of miss folded proteins), effectively down regulated apoptotic proteins (Caspase-3).

Phytochemical constituents and their radical scavenging and pro-inflammatory activities are the key sources to the antioxidant and anti-inflammatory potential of MR leaves and GL fruit rind to develop the drugs/nutraceuticals of antioxidant, gastroprotective and anti-inflammatory properties. These scientific validations confirm the ethno-therapeutic applications of MR and GL, signifying that the metabolites present in MRME and GLME can act as a phyto-complex facilitate to reduce the effects of the offensive agents (lipid peroxidation and ROS) and to fortify the defensive factors (proinflammatory agents) for the management of gastric ulcer. Pharmacologically active compounds from the MR (Bergenin and betulinic acid) and GL ought to be tested for the efficacy in the treatment of GU complications. Thus MRME and GLME could be act as a low cost valuable potential source of anti-ulcerogenic agents to meet sustainable development goals (SDGs) of the United Nations (UN).

Annexure-I

List of Compounds annotated with HR-LCMS and mZ Cloud as full match for GLME

Checked	Name (Postive ionization)	Formula	Annotation Source: mzCloud Search	Molecular Weight
TRUE	Bis(2-ethylhexyl) phthalate	C24 H38 O4	Full match	390.2755
TRUE	L-Cystine	C6 H12 N2 O4 S2	Full match	240.0242
TRUE	Octyl decyl phthalate	C26 H42 O4	Full match	418.3075
TRUE	Choline	C5 H13 N O	Full match	103.0998
TRUE	Ranitidine	C13 H22 N4 O3 S	Full match	314.14
TRUE	3-Hydroxypicolinic acid	C6 H5 N O3	Full match	139.0272
TRUE	Bromhexine	C14 H20 Br2 N2	Full match	373.998
TRUE	Dibutyl maleate	C12 H20 O4	Full match	228.1355
TRUE	cis,cis-Muconic acid	C6 H6 O4	Full match	142.0269
TRUE	Timolol	C13 H24 N4 O3 S	Full match	316.1554
TRUE	D-(+)-Pyroglutamic Acid	C5 H7 N O3	Full match	129.0429
TRUE	Betaxolol	C18 H29 N O3	Full match	307.2136
TRUE	17α-Ethynylestradiol	C20 H24 O2	Full match	296.1769
TRUE	Bis(2-ethylhexyl) phthalate	C24 H38 O4	Full match	390.2756
TRUE	Quinine	C20 H24 N2 O2	Full match	324.1824
TRUE	5-Hydroxymethyl-2-furaldehyde	C6 H6 O3	Full match	126.032
TRUE	L-Cystine	C6 H12 N2 O4 S2	Full match	240.0242
TRUE	cis,cis-Muconic acid	C6 H6 O4	Full match	142.0269
TRUE	Pyrrole-2-carboxylic acid	C5 H5 N O2	Full match	111.0322
TRUE	Didecyl phthalate	C28 H46 O4	Full match	446.3388
TRUE	Diethyl phthalate	C12 H14 O4	Full match	222.0885
TRUE	Ranitidine	C13 H22 N4 O3 S	Full match	314.1402
TRUE	Ranitidine	C13 H22 N4 O3 S	Full match	314.14
TRUE	Bis(2-ethylhexyl) phthalate	C24 H38 O4	Full match	390.2756
TRUE	Leucine	C6 H13 N O2	Full match	131.0949
TRUE	Adenine	C5 H5 N5	Full match	135.0548
TRUE	2-Methoxyresorcinol	C7 H8 O3	Full match	140.0477
TRUE	Bis(2,2,6,6-tetramethyl-4-piperidyl)sebacate	C28 H52 N2 O4	Full match	480.3942
TRUE	Bis(2-ethylhexyl) phthalate	C24 H38 O4	Full match	390.2756
TRUE	Dibutyl phthalate	C16 H22 O4	Full match	278.1505
TRUE	2-Methoxyresorcinol	C7 H8 O3	Full match	140.0477

TRUE	Didecyldimethylammonium	C22 H47 N	Full match	325.3694
TRUE	L-Pyroglutamic acid	C5 H7 N O3	Full match	129.0429
TRUE	5-Hydroxymethyl-2-furaldehyde	C6 H6 O3	Full match	126.032
TRUE	2-Aminonicotinic acid	C6 H6 N2 O2	Full match	138.0432
TRUE	L-Dopa	C9 H11 N O4	Full match	197.0691
TRUE	Caffeic acid	C9 H8 O4	Full match	180.0424
TRUE	2-Methoxyresorcinol	C7 H8 O3	Full match	140.0477
TRUE	D-Glucosamine	C6 H13 N O5	Full match	179.0795
TRUE	L-Glutamic acid	C5 H9 N O4	Full match	147.0534
TRUE	L-Serine	C3 H7 N O3	Full match	105.0427
TRUE	Bromhexine	C14 H20 Br2 N2	Full match	373.998
TRUE	2-Methoxyresorcinol	C7 H8 O3	Full match	140.0477
TRUE	2,6-Dimethoxyphenol	C8 H10 O3	Full match	154.0633
TRUE	Cetrimonium	C19 H41 N	Full match	283.3228
TRUE	Pyrrole-2-carboxylic acid	C5 H5 N O2	Full match	111.0322
TRUE	Diisodecyl phthalate	C28 H46 O4	Full match	446.3389
TRUE	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	C15 H22 O2	Full match	234.1616
TRUE	Thioridazine	C21 H26 N2 S2	Full match	370.1539
TRUE	Dipentyl phthalate	C18 H26 O4	Full match	306.1819
TRUE	5-Aminolevulinic acid	C5 H9 N O3	Full match	131.0585
TRUE	Pyrrole-2-carboxylic acid	C5 H5 N O2	Full match	111.0322
TRUE	Bis(2,2,6,6-tetramethyl-4-piperidyl)sebacate	C28 H52 N2 O4	Full match	480.3942
TRUE	cis,cis-Muconic acid	C6 H6 O4	Full match	142.0269
TRUE	4-Hydroxycoumarin	C9 H6 O3	Full match	162.0318
TRUE	Vitexin	C21 H20 O10	Full match	432.1048
TRUE	Dioctyl phthalate	C24 H38 O4	Full match	390.2756
TRUE	L-Norleucine	C6 H13 N O2	Full match	131.0949
TRUE	Pyrrole-2-carboxylic acid	C5 H5 N O2	Full match	111.0322
TRUE	7-Aminonitrazepam	C15 H13 N3 O	Full match	251.1053
TRUE	Urocanic acid	C6 H6 N2 O2	Full match	138.0432
TRUE	(1S)-1,5-Anhydro-2-O-(6-deoxy-α-L-mannopyranosyl)-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-6-yl]-D-glucitol	C27 H30 O14	Full match	578.163
TRUE	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	C9 H19 N O	Full match	157.1469
TRUE	Pyrrole-2-carboxylic acid	C5 H5 N O2	Full match	111.0322
TRUE	cis,cis-Muconic acid	C6 H6 O4	Full match	142.0269
TRUE	Guanine	C5 H5 N5 O	Full match	151.0496

TRUE	SDB-006 N-phenyl analog	C20 H22 N2 O	Full match	306.172
TRUE	4-Hydroxycoumarin	C9 H6 O3	Full match	162.0318
TRUE	DL-Stachydrine	C7 H13 N O2	Full match	143.0949
TRUE	6-Hydroxynicotinic acid	C6 H5 N O3	Full match	139.0273
TRUE	Betaxolol	C18 H29 N O3	Full match	307.2136
TRUE	Triphenylphosphine oxide	C18 H15 O P	Full match	278.0849
TRUE	3-Hydroxypicolinic acid	C6 H5 N O3	Full match	139.0272
TRUE	Erucamide	C22 H43 N O	Full match	337.3332
TRUE	Glipizide	C21 H27 N5 O4 S	Full match	445.1777
TRUE	Quinine	C20 H24 N2 O2	Full match	324.1824
TRUE	N-Methyl homarylamine	C11 H15 N O2	Full match	193.1106
TRUE	Atomoxetine	C17 H21 N O	Full match	255.1618
TRUE	Muramic acid	C9 H17 N O7	Full match	251.0998
TRUE	Oleamide	C18 H35 N O	Full match	281.2708
TRUE	4-Hydroxycoumarin	C9 H6 O3	Full match	162.0318
TRUE	Chlorhexidine	C22 H30 Cl2 N10	Full match	504.2039
TRUE	DL-Arginine	C6 H14 N4 O2	Full match	174.1119
TRUE	Guanine	C5 H5 N5 O	Full match	151.0496
TRUE	Benazol P	C13 H11 N3 O	Full match	225.0902
TRUE	Hexadecanamide	C16 H33 N O	Full match	255.2556
TRUE	Guanine	C5 H5 N5 O	Full match	151.0496
TRUE	Vitexin	C21 H20 O10	Full match	432.1046
TRUE	Ranitidine	C13 H22 N4 O3 S	Full match	314.14
TRUE	Baicalin	C21 H18 O11	Full match	446.0845
TRUE	Leucine	C6 H13 N O2	Full match	131.0949
TRUE	Docosanamide	C22 H45 N O	Full match	339.3484
TRUE	Diisobutylphthalate	C16 H22 O4	Full match	278.1505
TRUE	Diisobutylphthalate	C16 H22 O4	Full match	278.1505
TRUE	Nortriptyline	C19 H21 N	Full match	263.1665
TRUE	cis,cis-Muconic acid	C6 H6 O4	Full match	142.0269
TRUE	Dibutyl phthalate	C16 H22 O4	Full match	278.1505
TRUE	L-Cystine	C6 H12 N2 O4 S2	Full match	240.0242
TRUE	Scutellarin	C21 H18 O12	Full match	462.0799
TRUE	Epitestosterone	C19 H28 O2	Full match	288.2079
TRUE	Quinine	C20 H24 N2 O2	Full match	324.1824
TRUE	Oleamide	C18 H35 N O	Full match	281.2708
TRUE	cis-12-Octadecenoic acid methyl ester	C19 H36 O2	Full match	296.2701
TRUE	Oleamide	C18 H35 N O	Full match	281.2708
TRUE	1-Tetradecylamine	C14 H31 N	Full match	213.2458
TRUE	Noscapine	C22 H23 N O7	Full match	413.1466
TRUE	Neotame	C20 H30 N2 O5	Full match	378.2159

TRUE	Psychosine	C24 H47 N O7	Full match	461.3353
TRUE	5-Hydroxymethyl-2-furaldehyde	C6 H6 O3	Full match	126.032
TRUE	Betaine	C5 H11 N O2	Full match	117.0791
Negative ionization				
TRUE	3-Hydroxy-3-(methoxycarbonyl)pentanedioic acid	C7 H10 O7	Full match	206.0423
TRUE	Dodecyl sulfate	C12 H26 O4 S	Full match	266.1549
TRUE	3-Hydroxy-3-(methoxycarbonyl)pentanedioic acid	C7 H10 O7	Full match	206.0423
TRUE	Dodecyl sulfate	C12 H26 O4 S	Full match	266.1549
TRUE	4-Dodecylbenzenesulfonic acid	C18 H30 O3 S	Full match	326.1912
TRUE	Isocitric acid	C6 H8 O7	Full match	192.0267
TRUE	Dodecyl sulfate	C12 H26 O4 S	Full match	266.1549
TRUE	Citric acid	C6 H8 O7	Full match	192.0267
TRUE	Myristyl sulfate	C14 H30 O4 S	Full match	294.1863
TRUE	2-Furoic acid	C5 H4 O3	Full match	112.016
TRUE	Butylparaben	C11 H14 O3	Full match	194.094
TRUE	2-Furoic acid	C5 H4 O3	Full match	112.016
TRUE	L-(-)-Malic acid	C4 H6 O5	Full match	134.0214
TRUE	Dodecyl sulfate	C12 H26 O4 S	Full match	266.1549
TRUE	2-Furyl(5-hydroxy-1-benzofuran-3-yl)methanone	C13 H8 O4	Full match	228.0421
TRUE	Azelaic acid	C9 H16 O4	Full match	188.1047
TRUE	3-Hydroxyanthranilic acid	C7 H7 N O3	Full match	153.0425
TRUE	4-Oxoproline	C5 H7 N O3	Full match	129.0425
TRUE	3-Aminosalicylic acid	C7 H7 N O3	Full match	153.0425
TRUE	trans-Aconitic acid	C6 H6 O6	Full match	174.0161
TRUE	2,2-Dimethyl-3-hydroxy-3-(p-tolyl)propionic acid	C12 H16 O3	Full match	208.1097
TRUE	δ-Ribono-1,4-lactone	C5 H8 O5	Full match	148.0371
TRUE	trans-Aconitic acid	C6 H6 O6	Full match	174.0161
TRUE	Gluconic acid	C6 H12 O7	Full match	196.058
TRUE	16-Hydroxyhexadecanoic acid	C16 H32 O3	Full match	272.2349
TRUE	D-(+)-Galactose	C6 H12 O6	Full match	180.0632
TRUE	(1S)-1,5-Anhydro-2-O-(6-deoxy-α-L-mannopyranosyl)-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-6-yl]-D-glucitol	C27 H30 O14	Full match	578.1632
TRUE	Butylparaben	C11 H14 O3	Full match	194.094
TRUE	Azelaic acid	C9 H16 O4	Full match	188.1047

TRUE	2-Furoic acid	C5 H4 O3	Full match	112.016
TRUE	Luteolin	C15 H10 O6	Full match	286.0476
TRUE	δ-Ribono-1,4-lactone	C5 H8 O5	Full match	148.0371
TRUE	Glipizide	C21 H27 N5 O4 S	Full match	445.1781
TRUE	Butylparaben	C11 H14 O3	Full match	194.094
TRUE	L-Glutamic acid	C5 H9 N O4	Full match	147.053

Annexure-II

List of abbreviations:

AA: Acetic acid

ALDH: Acetaldehyde dehydrogenase

ANOVA: Analysis of variance

AP-1: Activator Protein-1

ASA: 5-aminosalicylic acid

BAO: Baseline acid output

BAX: Bcl-2 Associated X-protein

Bcl2: B-cell lymphoma 2

CAC: Colitis-associated cancer

CAT: Catalase

CE: collision energies

CGRP: Calcitonin Gene-Related Peptide

CINC-1: Cytokine-induced neutrophil chemoattractant-1

CMC: Carboxymethyl cellulose

CNS: Central nervous system

COX I: Cyclooxygenases I

COX II: Cyclooxygenases II

cPLA2: Cytosolic Phospholipase A2

DAB: Diaminobenzidine

DAI: Disease activity index

DNA: Deoxyribonucleic acid

DSS: Dextran sulfate sodium

DTNB: 5,5′-dithiobis-(2-nitrobenzoic acid)

dUTP: deoxynucleotide triphosphate

EC cells: Enterochromaffin cells

EGF: Epidermal growth factor

EP1: Prostaglandin E2 receptor 1

ESI: Electrospray ionization

eV: Electron volt

EVO: Evodiamine

FGPs: Fundic gland polyps

FVB/NJ: Friend Virus B/NIH Jackson

g: Gram

G-cells: Gastrin expressing cells

GC-MS: Gas chromatography–mass spectrometry

GIT: Gastro intestinal tract

GM: Gastric mucosa

GPx: Glutathione peroxide

GSH: Glutathione

GST: Glutathione S-transferase

GU: Gastric ulcer

GWM: Gastric wall mucus

H+/K+ ATPase: Hydrogen Potassium Adenosine Triphosphatase

$H_{2:}$ Hydrogen gas

HA: Hyaluronic acid

HB-EGF: Heparin-binding EGF-like growth factor

HCL: Hydrogen chloride

H&E: Hematoxylin and eosin

HR-LCMS: High Resolution Liquid Chromatograph Mass Spectrometer

HRP: Horseradish peroxidase

HSP: Heat shock protein

IBD: Inflammatory bowel disease

IEC: Intestinal epithelial cell

IgG: Immunoglobulin G

IHC: Immunohistochemistry

IIT: Indian Institute Technology

IL-17: Interleukin

IL1β: Interleukin-1β

IL-6: Interleukin-6

INOS: Inducible nitric oxide synthase

KA: Koreanaside A

KATP channel: ATP-sensitive potassium channel

kg: Kilogram

LPO: Lipid peroxidation

LTB4: Leukotriene B4

M: Molarity

MAPK-II: Mitogen-activated protein kinase II

mEq/L: Milliequivalents per lite

MDA: Malondialdehyde

mg: Milligram

mL: Milliliter

MMPs: Matrix metalloproteinases

MPO: Myeloperoxidase

MS2: Tandem mass spectrometry

$NaCO_3$: Sodium carbonate

NADH: Nicotinamide adenine dinucleotide

NBT: Nitroblue tetrazolium

NF-κB: Nuclear Factor Kappa B

nM: nano Mole

nmol: Nanomole

NO: Nitric oxide

NO_2: Nitrite

NO_3: Nitrate

NP-SH: Non-protein sulfhydryl

Nrf2: Nuclear factor erythroid 2-related factor 2

NSAIDs: Non–steroid anti-inflammatory drugs

NUD: Non-ulcer dyspepsia

p: Probability value

PBS: Phosphate Buffered saline

PCNA: Proliferating cell nuclear antigen

PG12: Prostaglandin I2

PGE2: Prostaglandin E2

PGs: Prostaglandins

pH: Potential of hydrogen

PMS: Phenazine methosulphate

PPI: Proton pump inhibitor

PUD: Peptic ulcer disease

RAS: Recurrent aphthous stomatitis

ROS: Reactive oxygen species

SAS: Sulfasalazine

SOD: Superoxide dismutase

STAT1/3: Signal transducer and activator of transcription 1/3

TBARS: Thiobarbituric acid reactive substances

TCA: Trichloroacetic acid

TCA: Trichloroacetic acid

TNBS: 2,4,6-Trinitrobenzensulfonic acid

TNF: Tumor necrosis factor

TUNEL: Terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling

UC: Ulcerative colitis

UI: ulcer index

USA: United States of America

VEGF: Vascular Endothelial Growth Factor

VIP: Vasoactive intestinal polypeptide

VWF: Von Willebrand factor

XO: xanthine oxidase

µg: Micorgram

5-HT: 5-hydroxytryptamine

5-LOX: arachidonate 5-lipoxygenase

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ABSTRACT

GASTROPROTECTIVE EFFECT OF METHANOL LEAF EXTRACT OF MALLOTUS ROXBURGHIANUS MULL.ARG. AND FRUIT EXTRACT OF GARCINIA LANCEIFOLIA ROXB. IN GASTRIC ULCERS IN WISTAR ALBINO RATS.

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ABSTRACT

GASTROPROTECTIVE EFFECT OF METHANOL LEAF EXTRACT OF MALLOTUS ROXBURGHIANUS MULL.ARG. AND FRUIT EXTRACT OF GARCINIA LANCEIFOLIA ROXB. IN GASTRIC ULCERS IN WISTAR ALBINO RATS.

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Thesis Title: Gastroprotective effect of methanol leaf extract of *Mallotus roxburghianus* Mull.Arg. and fruit extract of *Garcinia lanceifolia* Roxb. in gastric ulcers in Wistar albino rats.

Background:

Gastric ulcer (GU) disease is a well-known global health problem characterized by the presence of benign lesions/open sores of the gastric mucosa of stomach that might reach the muscularis mucosa or beyond (Escobedo-Hinojosa et al., 2018). Gastric ulcer is a one of the most prevalent worldwide gastrointestinal ailments and its prevalence is dominant in the Asian countries (Dutta et al., 2012). According to reports, GU has a 5–10% lifetime frequency in the general population and an annual incidence of 0.1-0.3 % (Lanas and Chan, 2017). Thus, GU is a crucial health issue affects an estimated 10% of the world's population and has a significant negative impact on millions of people's quality of life (Havens et al., 2018). Progression of GU results in haemorrhage (20%) and perforation (7%), affecting 87.4 million of the world population and resulted in 0.267 million deaths in 2015 (Wang et al., 2016). The peptic ulcer condition is extremely common among people worldwide, to the point where some academics have dubbed it the new emerging pandemic of the twenty-first century (Sumbul et al., 2011).

The pathogenesis of gastric ulcers is complex with lesions in mucus layer may be associated one or variety of internal and external factors. The GU results from an imbalance between various damaging/injurious and protective components (Gugliandolo et al., 2021). Aggressive/injurious factors includes endogenous pathogenic agents and events like elevated gastric hydrochloric acid, pepsin secretion, lipid peroxidation, inherited predisposition and excessive reactive oxygen species (ROS) production (Thorsen et al., 2013; Graham 2014,) and exogenous factors such as alcohol consumption, physical and psychological stress, tobacco and cigarette smoking, long-term use of non–steroid anti-inflammatory drugs(NSAIDs), antiplatelet agents (acetyl salicylic acid), immunosuppressive medications, serotonin reuptake inhibitors, pollutants, pesticides, heavy metals and gram-negative microaerophilic bacterial infection e.g. *Helicobacter pylori*(Ji et al., 2012; Mousavi

et al., 2020; De Araújo et al., 2021). While protective factors includes, mucosal barrier such as gastric wall mucus(GWM), mucus(mucin secretion) and bicarbonate production, biosynthesis of gastroprotective prostaglandin, nitric oxide (NO), growth factors, adequate blood flow, normal tissue microcirculation, surface phospholipids, and endogenous antioxidants (Al Batran et al., 2013; Farzaei et al., 2015). Mucin secretion and interaction of acid and pepsin are the main components to protect the GM from lesions and used as indicators to assess the GU complications (Al Batranet al., 2013).

A number of medications are used to treat stomach ulcers. The first optional medications used to treat dyspepsia and heartburn is antacids. This group is ineffective in stopping or treating stomach ulcers, but it can be helpful in two ways: either by protecting the stomach's mucosal layer by increasing bicarbonate and mucus secretions or by neutralizing acidity inside the stomach lumen. Antibiotics can be used to treat stomach ulcers brought on by an *H. pylori* bacterial infection. A class of medications known as H_2 blockers, which includes cimetidine and ranitidine, was the primary treatment for stomach ulcers (Feely and Wormsley, 1983). PPIs, or proton pump inhibitors, are the most often prescribed medication for stomach ulcers patient (Savarino et al., 2017). The PPIs that are currently approved for the treatment and prevention of ulcers include rabeprazole, lansoprazole, esomeprazole, and omeprazole (Strand et al., 2017). Each of these medications has several side effects, cost and limitations (Singh et al., 2018).

The lack of safe and effective treatments for this widespread health issue drives the search for new classes of chemicals that have strong activity and a favourable safety profile. Stomach disease and disorder, including gastric ulcers; have been treated with medicinal plants and their secondary metabolites. Several secondary metabolites with distinct gastroprotective modes of action have been found in medicinal plants with gastroprotective properties (Singh et al., 2018). It has also been suggested that secondary metabolites has strong anti-inflammatory, anti-apoptotic, anti-lipid peroxidation, and antioxidant properties contribute to their gastroprotective effects (Singh et al., 2018; Raish et al., 2021; Shipa et al., 2022). Given that herbal remedies are thought to be less harmful, affordable, efficient, and relatively less toxic, they are

safe to use in the treatment of ulcers. *Mallotus roxburghianus* Mull. Arg. (Euphorbiaceae) and *Garcinia lanceifolia* are important medicinal plants natively found in Mizoram, North East India. Both plants reported to be used in stomach related ailments traditionally. The ethanol extract of *M. roxburghianus* has been reported for its anti-diabetic and anti-oxidant activity (Lalhlenmawia et al., 2007; Roy et al., 2016).

Looking into the potential prospects of above mentioned ethno medicinal plants, the present work was undertaken with following

OBJECTIVES:

- Detection and identification of phyto-compounds in *M. roxburghianus* and *G. lanceifolia* by GC-MS/LC-MS.
- Evaluation of anti-ulcer and ulcer healing activities.
- Histopathological analysis and examine the extent of oxidative stress and the role of antioxidant enzymes in attenuation of gastric ulcer by *M. roxburghianus* and *G. lanceifolia*.
- Role of inflammatory and apoptotic genes and proteins in gastric ulcer formation by immunohistochemistry, immunoblotting/RT-PCR.

Material and Methods:

Plant Collection: Fresh *M. roxburghianus* leaves were gathered from Ngopa village, Champhai district (Saitual district), Mizoram (22.52 °N, 92.89°E) while Fresh fruits of *G. lanceifolia* were collected from Thingdawl village (24.1663 °N; 92. 6947 °E), Kolasib district, Mizoram, in May 2021. The plants were locally and botanically identified and validated as per Flora of Mizoram and sample species preserved in the herbarium of Mizoram University.

Phytochemical analysis: Phytochemical constituents of *M. roxburghianus* methanol leaf extract (MRME) and *G. lanceifolia* methanol fruit extract was qualitatively and quantitatively analysed by LC–MS/MS and HR-LCMS. Acute and sub-acute toxicity assay of MRME and GLME were performed.

Experimental design: Healthy adult Wistar albino male and female rats (After 24 h of fasting, the rats, n = 5) were pre-treated orally. C, control group, and UC, ulcer control group, fed with 0.5% CMC vehicle (5mL/kg); OM, omeprazole group administered orally with 20 mg/kg in 0.5% CMC; and MRME (M5, M10, M20, M50, M100 and M200) groups received orally with 5, 10, 20, 100 and 200 mg/kg methanol leaf extract of *M. roxburghianus*, respectively as a pretreatment while GLME200, GLME400, GLME600 groups received orally with 200, 400 and 600 mg/kg, of methanol fruit extract with 0.5% CMC respectively as a pre-treatment.

Measurement of gastric parameters such as gastric juice pH, acidity, and gastric wall mucus (GWM) were estimated. Hematoxylin and eosin (H& E) staining and PAS staining were performed to evaluate histopathology of stomach associated with selected treatments. Lipid peroxidation (MDA) and antioxidant enzymes activity of SOD, GST and NO oxide level were estimated in gastric tissues for treatments. All parameters were estimated as per standard protocols mentioned in the thesis.

Immunohistochemical localization and western blot analysis: Evaluation of gastric cellular proteins, such as COX I, COX II, BAX, BCL2, HSP70, Caspase-3, TNF-α and PCNA were performed for MRME while BAX, BCL2, HSP70, Caspase-3 were evaluated for GLME treatments. Evaluation of gastric apoptotic cells by TUNEL assay was performed for MRME. All proteins localization/regulation were estimated as per standard protocols mentioned in the thesis.

Results:

LC-MS/MS chromatogram profile of MRME highlighted the presence of diverse phytocompounds as bergenin, betulinic acid, gallic acid, hesperidin, tannic acid, myrcetin, luteolin, kaempferol, quercetin, protocatechuic acid, hesperitin, gentisic acid, naringenin, ellagic acid, chlorogenic acid, rutin, genistein, chrysin and coumarin. While GLME is rich in amino acids (5-Aminolevulinic acid, L-Glutamic acid, L-Dopa, Leucine, L-Cystine, L-Serine, L-Glutamic acid and 3-Hydroxyanthranilic acid), organic acids (Pyrrole-2-carboxylic acid, L-(-)-Malic acid and Citric acid), fatty acids and their derivatives (Hexadecanamide, oleamide and 16-Hydroxyhexadecanoic acid), flavonoids (Vitexin, (1S)-1,5-Anhydro-2-O-(6-deoxy-

α-L-mannopyranosyl)-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-6-yl]-D-glucitol, Scutellarin, Luteolin) and courmarin (4-Hydroxycoumarin).

The MRME even at higher doses of 1000 mg/kg and 3000 mg/kg were found to be safe while higher doses of GLME (1000 mg/kg, 2000 mg/kg and 3000 mg/kg) found to be toxic as evaluated by acute toxicity assay and histopathological observation of organs such liver, kidney and spleen. Sub-acute toxicity assay of GLME found that lower doses (50 mg/kg, 100 mg/kg and 200 mg/kg) found to be safe. Dose of 5 ml/kg of ethanol is found to induce ulcer in the rats. Gastroprotective effect of GLME neither does not follow dose dependent activity, compared high dose (600 mg/kg) lower dose 400 mg/kg was found more effective while MRME followed dose dependent anti-ulcer activity with higher dose 200 mg/kg was most effective.

MRME exerted strong exerted dose dependent gastroprotective against ethanol induced gastric injury/ulcer via amelioration gastric juice pH, acidity, gastric wall mucus, eosinophils and mast cells. MRME found to significantly ($p<0.00l$) down regulated lipid peroxidation (MDA) and restored/up regulated the antioxidant markers such as SOD and GST and NO in ethanol induced MRME pre-treated rats.

Similar to MRME, GLME also exerted gastroprotective against ethanol induced gastric injury/ulcer via amelioration gastric juice pH, acidity, and strengthening of gastric wall mucus. GLME found to significantly ($p<0.00l$) down regulated lipid peroxidation (MDA) and restored the antioxidant markers such as SOD and GST and NO in ethanol induced GLME pretreated rats with maximum protection was recorded at 400 mg/kg dose. Both MRME and GLME significantly decreased the ulcer index and enhanced percentage ulcer inhibition index.

The MRME has effectively exerted gastro cell protective through maintenance/up regulation following regulatory gastric proteins such as COX-I, COX-II(responsible for PGE & NO synthesis, gastric flow), BCL2(anti-apoptotic), PCNA(DNA replication & cell cycle regulation marker) and HSP70(prevent cell from aberrant aggregation and refolding of miss folded proteins), effectively down regulated apoptotic proteins (BAX and Caspase-3). Tunnel assay validated DNA protective property of MRME. All the results were found to be statistically significant

($p<0.00l$). The number of apoptotic cells (brown spots) was significantly greater in the gastric mucosa of ethanol induced group compared to the control group ($p<0.001$). We found there is a significant decrease in the number of apoptotic cells in groups rats pre-treated with MRME (100mg/kg and 200mg/kg doses) compared to control group($p<0.001$). Thus, the assay clearly indicates the DNA protecting ability in the gastric mucosa of the MRME extract.

GLME at the dose of 400 mg/kg; has effectively exerted gastro cell protective through maintenance/up regulation BCL2 (anti-apoptotic), HSP70 (prevent cell from aberrant aggregation and refolding of miss folded proteins), effectively down regulated apoptotic proteins (Caspase-3). All the results were found to be statistically significant ($p<0.00l$).

Conclusions:

Phytochemical constituents and their radical scavenging activities are the key sources to the antioxidant and anti-inflammatory potential of MR leaves and GL fruit rind to develop the drugs/nutraceutical of antioxidant, gastroprotective and anti-inflammatory properties. These scientific validations confirm the ethnotherapeutic applications of MR and GL, signifying that the metabolites present in MRME and GLME can act as a phyto-complex facilitate to reduce the effects of the offensive agents (lipid peroxidation and ROS) and to fortify the defensive factors (mucin, NO, anti-apoptotic) for the management of gastric ulcer. Pharmacologically active compounds from the MR (Bergenin and betulinic acid) and GL ought to be tested for the efficacy in the treatment of GU complications. Thus, MRME and GLME could be act as a low-cost valuable potential source of anti-ulcerogenic agents to meet sustainable development goals (SDGs) of the United Nations (UN).