

EVALUATE THE ANTI-ULCER ACTIVITY OF THE ETHANOLIC EXTRACT OF TORENIA CRUSTACEA LEAVES USING SUITABLE EXPERIMENTAL MODELS

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ABSTRACT

Background: Gastric ulceration is a common gastrointestinal illness related through oxidative stress, swelling and disturbance of the stomach mucosal barrier. Medicinal plants containing bioactive phytochemicals have attracted attention as potential sources of gastroprotective agents. **Objective:** The present investigation aimed to investigate the phytochemical composition, gastroprotective potential and antioxidant action of the ethanolic leaf extract of *Torenia crustacea* against ethanol-induced gastric ulceration into experimental animals. **Methods:** Preliminary phytochemical screening and thin-layer chromatography (TLC) were performed to investigate the chemical ingredients of the extract. Experimental animals has divided into six groups comprising normal control, ulcer control, standard control (ranitidine 50 mg/kg) and three extract-treated groups receiving 100, 200 and 300 mg/kg, respectively. Gastroprotective activity was assessed through ulcer index, percentage gastroprotection, gastric lesion score and lesion area. Oxidative stress markers, MDA, SOD, CAT and GSH, were evaluated. Gastric tissues had been also observed Histopathological. **Results:** In the illustrative dataset, the extract exhibited a dose-dependent gastroprotective pattern, with ulcer inhibition values of 30.76%, 50.60% and 65.62% at 100, 200 and 300mg/kg, respectively, compared with 74.94% for ranitidine. Increasing extract doses were associated with reduced gastric lesion severity and decreased MDA levels, alongside improvements in SOD, catalase and GSH levels. Representative histopathological illustrations showed progressively better preservation of gastric mucosal architecture at higher extract doses. **Conclusion:** The illustrative findings suggest that *Torenia crustacea* ethanolic leaf extract may have gastroprotective and antioxidant potential, possibly associated with its phytochemical constituents. Further experimental validation and identification of active compounds are necessary to establish its therapeutic significance.

KEYWORDS: *Torenia crustacea*, gastric ulcer, gastroprotective activity, oxidative stress, antioxidant enzymes, phytochemical screening, ethanol-induced ulceration.

Chapter 1

INTRODUCTION

1.1 Background of Peptic Ulcer Disease

Peptic ulcer disease (PUD) is a common illness of the upper stomach tract characterized through the development of a local defect or ulceration in the mucosal lining of the stomach or proximal part of the duodenum. The lesion improves when the normal defensive mechanisms of the stomach mucosa become insufficient to withstand the harmful action of gastric acid, pepsin, bile salts, reactive oxygen species,

inflammatory mediators, and other injurious factors. Under physiological conditions, the gastric mucosa is continuously visible to potentially harmful luminal contents, yet it remains intact because of a complex network of defense and repair mechanisms. Peptic ulceration happens once the equilibrium between aggressive and protective issues is disturbed for a sufficient period to produce mucosal injury and delay normal healing.^[1]

The stomach possesses a highly specialized mucosal barrier that protects the underlying epithelium from gastric acid and digestive enzymes. Mucus and bicarbonate secretion creates a protective microenvironment close to the epithelial surface, while adequate mucosal blood flow supplies oxygen and nutrients and facilitates the removal of hydrogen ions and toxic metabolites. Gastric epithelial cells also undergo continuous renewal, allowing damaged cells to be replaced rapidly. When these defensive mechanisms are compromised, even physiological levels of gastric acid and pepsin may contribute to the growth and headway of mucosal lesions.^[2]

The development of peptic ulcer disease is therefore not simply a consequence of excessive gastric acid secretion. It is better understood as a multifactorial pathological process involving the interaction of acid-peptic activity, impaired mucosal defense, inflammation, oxidative stress, altered blood flow, delayed epithelial repair, and environmental or pharmacological factors. Gastric acid remains an important contributor to ulcer formation because hydrogen ions can penetrate a damaged epithelial barrier and cause cellular injury. Pepsin, a proteolytic enzyme activated in an acidic environment, can further aggravate the damage by degrading proteins within the mucosal tissue. However, ulceration may occur even when acid secretion is not markedly increased if mucosal defense and repair processes are substantially impaired.^[3]

One of the major recognized reasons of peptic ulcer illness is infection with *Helicobacter pylori*. This microorganism is capable of colonizing the gastric mucosa and establishing persistent inflammation. Its ability to survive in the acidic gastric environment is associated with several adaptive mechanisms, including urease-mediated modification of the local environment. Persistent infection can alter epithelial integrity, stimulate inflammatory responses, and interfere with normal mucosal defense. The inflammatory reaction associated with *H. pylori* infection can promote tissue injury through the release of cytokines, chemokines, reactive oxygen species, and other inflammatory mediators.^[4]

Non-steroidal anti-inflammatory drugs (NSAIDs) characterize another significant danger issue for peptic ulceration. Medications like as aspirin, ibuprofen, naproxen, and related agents can interfere with prostaglandin synthesis by inhibiting cyclooxygenase enzymes. Because prostaglandins contribute to saliva and bicarbonate production, mucosal plasma flow, epithelial healing, and other protective processes, their reduction can weaken the gastrointestinal barrier. In addition to systemic prostaglandin depletion, some NSAIDs can produce direct topical frustration of the gastric mucosa. The combined effects may increase susceptibility to stomach injury, particularly in individuals receiving

prolonged treatment, higher doses, or multiple ulcerogenic medications.^[5]

Alcohol consumption can also contribute to gastric mucosal damage. Ethanol may disturb the integrity of the gastric epithelial barrier, alter membrane properties, increase mucosal permeability, and promote oxidative injury. Experimental administration of ethanol is frequently used to investigate gastroprotective activity because it can produce rapid and reproducible gastric lesions through a combination of mechanisms. These include disruption of the mucus barrier, vascular alterations, oxidative stress, inflammatory responses, and direct epithelial injury. The ethanol-induced ulcer model is therefore useful for evaluating whether a test substance can protect the gastric mucosa against acute chemical injury.^[6]

Psychological and physiological stressors may influence gastric ulcer development through neuroendocrine and vascular mechanisms. Severe stress can alter gastric motility, emission, mucosal plasma flow, and inflammatory responses. Stress-associated changes in autonomic activity and glucocorticoid signaling may further influence the integrity and repair capacity of the gastric mucosa. Although stress alone does not explain all cases of peptic ulcer disease, it can aggravate existing gastrointestinal vulnerability and may contribute to the severity of mucosal injury under particular conditions.^[7]

Smoking is another recognized factor associated with gastrointestinal ulcer risk and impaired ulcer healing. Tobacco smoke contains numerous biologically active compounds that can influence gastric physiology, mucosal blood flow, oxidative status, and tissue repair. Smoking may also interact with other risk issues, with *H. pylori* infection and NSAID use, potentially increasing susceptibility to gastric injury. Similarly, dietary habits, excessive intake of irritating substances, alcohol exposure, and other lifestyle factors may influence gastrointestinal health, although their contribution can vary considerably between individuals.

Oxidative stress has emerged as an important component of gastric mucosal injury. Under normal physiological conditions, reactive oxygen species are generated throughout cellular metabolism and are controlled by endogenous antioxidant systems. During pathological conditions, lipid peroxidation can produce secondary reactive molecules that further damage cellular structures and compromise the integrity of the gastric mucosal barrier.^[8]

The antioxidant defense system is therefore closely connected with the ability of gastric tissue to resist injury. Reduced glutathione is another important component of cellular antioxidant defense and participates in the neutralization of responsive classes and conservation of cellular redox balance. During ulcerative injury, depletion of endogenous antioxidants

may accompany an increase in lipid peroxidation products such as malondialdehyde. Measurement of these parameters can consequently provide useful information regarding the oxidative component of gastric injury and the potential antioxidant contribution of a gastroprotective intervention.^[9]

Inflammation is also closely related by the growth and progression of stomach mucosal damage. Injury to gastric epithelial cells can stimulate the release of inflammatory mediators and promote recruitment of immune cells into the affected tissue. Cytokines like as cancer necrosis factor alpha and interleukin6 might participate in the amplification of inflammatory responses, while signaling pathways involving nuclear factor kappa B can regulate the expression of several inflammatory genes. Excessive or persistent activation of these pathways may increase tissue injury and interfere with mucosal recovery. Consequently, suppression of excessive inflammation may represent an important component of the gastroprotective action of pharmacological or plant-derived interventions.^[10]

The gastric mucosa also possesses an important capacity for rapid repair. Following superficial injury, surviving epithelial cells migrate toward damaged areas and restore continuity of the epithelial layer. Cellular proliferation subsequently replaces lost cells and contributes to the restoration of normal tissue architecture. Adequate blood supply is essential for these processes because mucosal perfusion provides oxygen and nutrients and supports the removal of potentially harmful metabolites. Disturbances in microcirculation can therefore increase the severity of injury and impair healing.^[11]

Several endogenous mediators participate in maintaining gastric mucosal integrity. Prostaglandins are particularly important because they regulate mucus and bicarbonate emission, uphold mucosal circulation, support epithelial restitution, and modulate inflammatory responses. Nitric oxide also contributes to mucosal protection through effects on blood flow, vascular tone, mucus secretion, and cellular defense. Growth factors and other signaling molecules may additionally contribute to epithelial proliferation, angiogenesis, and tissue repair. A substance with gastroprotective potential may therefore act through one or several of these pathways rather than through acid suppression alone.^[12]

Clinically, peptic ulcer disease may present with upper abdominal discomfort, burning or gnawing epigastric pain, nausea, bloating, early satiety, or other nonspecific gastrointestinal symptoms. Some individuals may remain asymptomatic until complications develop. Serious complications include gastrointestinal bleeding, perforation, penetration into adjacent tissues, and gastric outlet obstruction. Upper gastrointestinal bleeding can manifest as hematemesis or melena and may become life-threatening if substantial blood loss occurs. Perforation is another severe complication in which an

ulcer penetrates the gastrointestinal wall and causes leakage of gastric or intestinal contents into the peritoneal cavity. These complications emphasize the importance of effective prevention, early diagnosis, and appropriate treatment.^[13]

The management of peptic ulcer illness depends on its underlying reason and clinical severity. Acid-suppressive medicines, particularly proton pump inhibitors, are central components of treatment. H₂-receptor antagonists and mucosal protective agents may also be used in selected situations. In patients with *H. pylori* infection, eradication therapy involving appropriate combinations of antimicrobial agents and acid suppression is an essential component of management. In NSAID-associated ulceration, discontinuation or modification of the offending drug, when clinically possible, together with appropriate gastroprotective therapy may be required. Although currently available therapies are effective for many patients, recurrence, treatment failure, antimicrobial resistance, adverse effects, drug interactions, and the need for prolonged treatment remain relevant clinical considerations.^[14]

These limitations have encouraged continued investigation into naturally occurring substances with gastroprotective and anti-ulcer properties. Therapeutic floras have historically been utilized in old-style healthcare systems for the management of gastrointestinal discomfort, inflammation, and digestive disorders. Some of these compounds have demonstrated antioxidant, anti-inflammatory, antimicrobial, cytoprotective, and tissue-repair-related activities. Such properties provide a rational basis for investigating medicinal plants as potential sources of gastroprotective agents.^[15]

The potential anti-ulcer effects of plant extracts may involve several complementary mechanisms. A plant-derived preparation may reduce gastric acid secretion, strengthen the mucus barrier, increase bicarbonate production, preserve mucosal blood flow, enhance endogenous antioxidant defenses, inhibit excessive inflammatory signaling, or promote epithelial regeneration. Flavonoids and phenolic compounds, for example, are frequently investigated because of their free-radical-scavenging and anti-inflammatory properties. Tannins may contribute to mucosal protection through interactions with proteins and formation of a protective layer, while other phytoconstituents may influence gastric secretion, inflammatory signaling, or tissue repair. However, the biological activity of a plant extract cannot be attributed to a single phytochemical constituent without appropriate experimental evidence.^[16]

In this context, investigation of less extensively studied medicinal plants may provide opportunities for identifying new sources of gastroprotective compounds. *Torenia crustacea* is of interest as a plant-derived

candidate for pharmacological investigation. Evaluation of its ethanolic leaf extract may provide information regarding the presence of bioactive phytoconstituents and their possible contribution to protection against gastric mucosal injury. Ethanolic extraction is particularly useful for recovering a broad range of moderately polar phytochemicals, although the actual composition and biological activity of an extract depend on plant material, geographical origin, extraction conditions, solvent composition, and other methodological factors.^[18]

The assessment of the anti-ulcer potential of *Torenia crustacea* should therefore extend beyond simple observation of ulcer reduction. A comprehensive evaluation can include preliminary phytochemical screening, estimation of phenolic and flavonoid contents, antioxidant assessment, determination of gastric pH and acidity, measurement of ulcer index and percentage protection, evaluation of oxidative stress markers, and histopathological examination of gastric tissues.^[19]

The study of medicinal plant extracts in experimental ulcer models can consequently contribute to the broader understanding of natural gastroprotective agents. Demonstration of biological activity, however, represents an initial stage of investigation and should be followed by appropriate phytochemical characterization, dose-response studies, safety assessment, mechanistic investigations, and further pharmacological evaluation. Identification of active constituents and clarification of their molecular targets would be particularly valuable for determining whether a plant extract has potential for future development as a standardized therapeutic or supportive gastroprot.^[20]

1.2 Definition of Gastric/Peptic Ulcer

A peptic ulcer was a restricted deficiency in the mucosal lining of the abdominal tract that develops at sites exposed to gastric acid and pepsin. It is characterized by disruption of the mucosal superficial with tissue loss extending beyond the superficial epithelial layer. Peptic ulcers most commonly occur in the stomach and proximal duodenum and are therefore generally secret as gastric or duodenal ulcers. The development of an ulcer reflects a pathological disparity among hostile factors, such as gastric acid, pepsin, reactive oxygen species, bile acids, and certain drugs, and the protective mechanisms responsible for maintaining gastrointestinal mucosal integrity.^[21]

A gastric ulcer refers specifically to an ulcerative lesion that develops within the stomach. Gastric mucosal injury occurs when the protective barrier of the stomach becomes inadequate to resist damaging influences. Disturbance of one or more of these mechanisms can increase mucosal susceptibility to acid-peptic injury and may ultimately result in erosion followed by deeper ulceration.^[22]

A peptic ulcer, in the broader sense, includes ulcerative lesions occurring in regions of the gastrointestinal tract that are exposed to gastric acid and peptic activity. The term is commonly used to encompass both gastric and duodenal ulcers. Although acid and pepsin contribute substantially to ulcer formation, peptic ulcer disease is not necessarily associated with excessive acid secretion. Instead, ulcer development frequently results from impairment of mucosal defense, inflammation, oxidative stress, infection, drug exposure, or a combination of these factors.^[23]

At the microscopic level, ulceration involves loss of the surface epithelium followed by damage to deeper mucosal structures. Depending on the severity and duration of injury, the lesion may extend through the mucosa and submucosa and can involve deeper layers of the gastrointestinal wall. Persistent inflammation may interfere with tissue repair and delay restoration of normal mucosal architecture.^[24]

1.3 Epidemiology and Prevalence of Peptic Ulcer Illness

PUD remnants a significant stomach disorder affecting populations across different geographical regions and socioeconomic groups. Although substantial improvements have occurred in the diagnosis, deterrence, and cure of ulcer illness, peptic ulceration continues to impose a considerable health burden because of its potential to cause gastrointestinal bleeding, perforation, obstruction, recurrent symptoms, hospitalization, and loss of productivity. The epidemiological characteristics of PUD have changed considerably over the past several decades, particularly following the recognition of *Helicobacter pylori* as a major etiological factor and the widespread use of NSAIDs, protonpump inhibitors, and *H. pylori* eradication therapies.^[25]

The prevalence and incidence of PUD differ considerably according to age, sex, geographical location, socioeconomic conditions, healthcare accessibility, and exposure to established risk factors. Historically, peptic ulcer disease was more frequently observed in middleaged and elder grown person, with a greater burden generally reported among males. However, changes in population demographics, increasing life expectancy, widespread use of ulcerogenic medications, and differences in *H. pylori* prevalence have altered the epidemiological pattern of the disease. Contemporary epidemiological analyses continue to demonstrate differences between males and females, with men generally experiencing a higher burden of PUD than women across many age groups.^[26]

Earlier systematic evidence suggested that the annual occurrence of physician-diagnosed PUD was approximately 0.10–0.19%, while estimates founded on hospitalization data ranged from 0.03–0.17%. The reported oneyear occurrence founded on physician

analysis ranged from approximately 0.12–1.50%. Considerable variation among studies was attributed to differences in study populations, diagnostic approaches, healthcare utilization, and geographical factors. The same systematic review also reported an overall decline in PUD incidence and prevalence in several populations, which was considered to be associated, at least in part, with reductions in *H. pylori*-associated ulcer disease and improvements in medical management.^[27]

Large-scale Global Burden of Disease (GBD) analyses provide further evidence regarding the worldwide distribution of PUD. According to the GBD 2019 analysis, approximately 8.09 million prevalent cases of PUD were estimated globally in 2019, compared with approximately 6.43 million cases in 1990. Thus, the absolute number of prevalent cases increased substantially over this period, largely reflecting population growth and ageing. In contrast, the global age-standardized prevalence ratio declined from approximately 143.4 per 100,000 population in 1990 to 99.4 per 100,000 population in 2019. Similarly, the global age-standardized incidence rate decreased during the same period, indicating an overall reduction in the population-level risk after adjustment for changes in population age structure.^[28]

More recent evidence from the GBD 2021 study provides an updated picture of the global burden. Between 1990 and 2021, the absolute number of PUD incidence and prevalence cases increased by approximately 11.1% and 8.8%, respectively. However, the corresponding age-standardized incidence and prevalence rates decreased by approximately 40.3% and 41.1%, respectively. This difference between absolute numbers and standardized rates is epidemiologically important: although the total number of people affected remains substantial, the underlying population-level burden has generally declined after accounting for demographic changes.^[29]

The epidemiological burden of PUD is not evenly distributed across the world. Socioeconomic and geographical disparities remain evident, with several analyses demonstrating a relatively higher age-standardized burden in low- and middle-socioeconomic settings. Such geographical differences may be related to variation in *H. pylori* infection, sanitation, nutritional conditions, NSAID exposure, smoking, healthcare access, socioeconomic status, and availability of diagnostic and therapeutic services.^[30]

India represents an important population for understanding the epidemiology of peptic ulcer disease because of its large population, regional socioeconomic variation, and historically substantial burden of gastrointestinal disorders. The epidemiology of PUD in India has undergone noticeable changes over time. A long-term endoscopic study conducted in India evaluated more than 30,000 patients with dyspeptic symptoms

across several periods between 1988 and 2008. Overall, 7.8% of the evaluated patients were diagnosed with PUD. During the study period, the frequency of duodenal ulcer decreased from approximately 12% to 2.9%, while gastric ulcer frequency decreased from approximately 4.5% to 2.7%. The decline was more pronounced for duodenal ulcer disease, resulting in a substantial reduction in the duodenal-to-gastric ulcer ratio.^[31]

The observed decline in PUD in several populations is likely multifactorial. Improved sanitation and living conditions may have reduced transmission of *H. pylori*, while improved diagnostic methods and effective eradication regimens have contributed to better control of infection-associated ulcer disease. The widespread availability of proton pump inhibitors and other acid-suppressive agents has also improved ulcer healing. At the same time, the increasing use of aspirin and NSAIDs, particularly among ageing populations and individuals with cardiovascular, musculoskeletal, and inflammatory conditions, remains an important concern. Therefore, the epidemiological transition of PUD should not be interpreted as complete elimination of the disease; rather, the relative contribution of different risk factors appears to have changed over time.^[32]

Age is another important determinant of PUD occurrence and clinical outcomes. The risk of ulcer disease and its complications generally increases with advancing age, particularly because older individuals are more likely to use aspirin and NSAIDs and may have multiple comorbidities. Age-related changes in mucosal defense, tissue repair, drug metabolism, and vascular function may further increase susceptibility to gastrointestinal injury. Older individuals may also experience more severe consequences following ulcer complications, particularly gastrointestinal bleeding. Recent epidemiological analyses have consequently identified age as an important factor influencing the distribution and severity of PUD.^[33]

Sex-related differences have also been reported in the epidemiology of PUD. Global burden analyses indicate that males generally have higher incidence and prevalence rates than females across many age categories. The difference may reflect variations in smoking, alcohol exposure, occupational stress, medication use, *H. pylori* infection patterns, and other biological or behavioral factors. However, the magnitude of the sex difference varies among populations, and increasing exposure to NSAIDs and aspirin among women may influence the epidemiological pattern in particular age groups.^[34]

The clinical importance of PUD is strongly related to its complications. Gastrointestinal bleeding and perforation are among the most serious complications and may require emergency medical or surgical intervention. Epidemiological studies have demonstrated substantial geographical variation in the incidence of complicated

ulcer disease. A methodical evaluation of population-based studies reported annual incidence rates of all PUD ranging from 23.9 to 141.8 cases per 100,000 persons across selected countries. For bleeding PUD, reported annual incidence ranged from 8.3 to 72.5 per 100,000, while perforated PUD ranged from 2.2 to 4.4 per 100,000 in the reviewed populations. These variations emphasize the influence of population characteristics, healthcare systems, risk-factor exposure, and study methodology on reported disease burden.^[35]

An important epidemiological feature of PUD is the distinction between absolute case numbers and age-standardized rates. Global population growth and ageing can increase the total number of individuals living with PUD even when the underlying risk of disease is decreasing. The GBD studies demonstrate this phenomenon clearly: the absolute number of prevalent cases increased between 1990 and 2019, whereas the age-standardized prevalence rate decreased substantially. Therefore, both absolute numbers and standardized rates should be considered when interpreting the epidemiological significance of PUD and when planning healthcare resources.^[36]

Recent epidemiological research also indicates that the global burden of PUD is likely to continue changing as population structures, healthcare access, medication utilization, smoking patterns, and *H. pylori* prevalence evolve. Although age-standardized rates have generally declined, millions of individuals continue to experience PUD worldwide, and the disease remains clinically relevant because of its potentially severe complications. The persistence of PUD, particularly in populations with limited healthcare resources, highlights the need for effective prevention, early diagnosis, appropriate treatment, and continued investigation of novel gastroprotective approaches.^[37]

1.4 Types of Peptic Ulcers

When stomach acid and pepsin come into contact with certain parts of the digestive tract, it can cause mucosal sores called peptic ulcers. Although ulcers in the stomach and duodenum are the most common signs of peptic ulcer disease, other adverse events, medications, systemic illnesses, or physiological stress can also cause ulcerative lesions. The classification of peptic ulcers can therefore be based on their anatomical location as well as their underlying cause. The major types relevant to experimental and clinical studies include gastric ulcer, duodenal ulcer, stress-induced ulcer, and drug-induced ulcer [38].

1.4.1 Gastric Ulcer

A gastric ulcer is a localized ulcerative lesion that develops in the mucosal lining of the stomach. It occurs when gastric mucosal defensive mechanisms are unable to adequately counteract inflammatory mediators, alcohol, *Helicobacter pylori*, or ulcerogenic drugs. Gastric ulcers may occur in different regions of the

stomach, although they are commonly observed along the lesser curvature. The pathogenesis of gastric ulcer involves disruption of the mucosal barrier, epithelial injury, impaired mucus and bicarbonate emission, reduced mucosal plasma movement, oxidative stress, inflammation, and delayed epithelial repair. Gastric acid and pepsin can penetrate the damaged mucosa and aggravate tissue injury. At the same time, depletion of endogenous antioxidant defenses may increase oxidative damage to cellular membranes and proteins.^[39]

H. pylori infection and NSAID exposure are important etiological factors in gastric ulceration. *H. pylori* can produce persistent gastric inflammation and interfere with mucosal defense, whereas NSAIDs can reduce prostaglandin synthesis and thereby compromise mucus creation, bicarbonate emission, mucosal plasma movement, and epithelial repair. Clinically, stomach ulcer may present with epigastric discomfort, burning or aching pain, nausea, early satiety, bloating, or other dyspeptic symptoms. However, some patients may remain asymptomatic until complications such as bleeding or perforation occur. In experimental pharmacological studies, gastric ulcer models are frequently used to assess the gastro protective potential of drugs and medicinal plant extracts.^[40]

1.4.2 Duodenal Ulcer

A duodenal ulcer is an ulcerative lesion that improves into the mucosa of the duodenum, particularly in the proximal duodenum. It is closely associated with disturbances in acid-peptic activity and mucosal defense. Excessive exposure of the duodenal mucosa to gastric acid, together with impaired bicarbonate-mediated neutralization and other protective mechanisms, can contribute to tissue injury. *H. pylori* infection is an important contributor to duodenal ulcer development. Infection can alter gastric physiology and inflammatory responses and may increase the delivery of acid into the duodenum. In susceptible individuals, this increased acid load can exceed the protective capacity of the duodenal mucosa. NSAIDs, smoking, genetic susceptibility, and other environmental factors may also contribute to disease development.^[41]

The duodenum possesses several protective mechanisms, including secretion of bicarbonate, mucus formation, adequate mucosal blood flow, epithelial restitution, and prostaglandin-mediated defense. Failure of these mechanisms may permit acid and pepsin to cause progressive mucosal damage. Patients with duodenal ulcers commonly experience epigastric pain or burning discomfort that may show a relationship with food intake. In some cases, pain may occur several hours after eating or during the night. As with gastric ulcer, serious complications include gastrointestinal bleeding and perforation.^[42]

1.4.3 Stress-Induced Ulcer

Stress-induced ulcers are acute gastric or gastroduodenal mucosal lesions associated with plain physical pressure. They are mainly relevant in critically ill patients and may occur in association with severe trauma, burns, major surgery, sepsis, neurological injury, respiratory failure, or other serious systemic conditions. Stress-related mucosal damage is distinct from ordinary psychological stress and is strongly associated with severe physiological disturbances. A major mechanism involved in stress-induced gastric injury is impairment of gastric mucosal blood flow. Severe physiological stress can cause redistribution of blood circulation away from the gastrointestinal tract, subsequent in reduced oxygen and nutrient distribution to the mucosa. Ischemia can compromise epithelial integrity and reduce the ability of the tissue to withstand acid-peptic injury.^[43]

Stress may also influence gastric secretion, gastrointestinal motility, inflammatory signaling, oxidative balance, and neuroendocrine responses. Reduced mucosal perfusion together with increased oxidative stress can promote epithelial damage and delay mucosal repair. Reactive oxygen species generated during ischemia and subsequent reperfusion may further enhance lipid peroxidation and cellular injury. Experimental stress-induced ulcer models are commonly employed in pharmacological research to investigate the ability of test compounds or plant extracts to protect gastric tissue against stress-related mucosal damage. These models are particularly useful for studying the interaction between stress, oxidative injury, mucosal blood flow, and endogenous protective mechanisms.^[44]

1.4.4 Drug-Induced Ulcer

Drug-induced ulcers are gastrointestinal mucosal lesions caused or aggravated by pharmacological agents. Among the various medications associated with ulcer development, NSAIDs are particularly important. Commonly used NSAIDs include aspirin, ibuprofen, naproxen, diclofenac, and related drugs. These agents can produce gastric injury through both systemic and local mechanisms. The principal systemic mechanism involves inhibition of cyclooxygenase-mediated prostaglandin synthesis. Suppression of prostaglandin production therefore weakens several components of the gastric defense system simultaneously.^[45]

NSAIDs may also produce direct topical injury to the gastric epithelium. Their interaction with the mucosal surface can increase epithelial permeability and facilitate penetration of hydrogen ions into the underlying tissue. This effect can be accompanied by mitochondrial dysfunction, oxidative stress, inflammatory activation, and cellular injury.

The risk of drug-induced ulceration is influenced by several factors, including dose, duration of treatment, age, history of ulcer disease, concomitant use of corticosteroids or antithrombotic medicines, *H. pylori*

infection, smoking, and other comorbidities. Older individuals may be particularly vulnerable because of increased use of NSAIDs and aspirin and the presence of multiple risk factors. Drug-induced ulcer models, particularly NSAID-induced gastric ulcer models, are widely used in experimental pharmacology. Agents such as aspirin or indomethacin can induce reproducible gastric mucosal lesions in laboratory animals and are therefore useful for evaluating the gastro protective and antiulcer properties of test substances.^[46]

1.5 Etiology and Risk Factors of Peptic Ulcer Disease

PUD was a multifactorial gastrointestinal disorder that develops due to an imbalance between destructive issues, such as gastric acid, pepsin, reactive oxygen species, bile reflux and the protective mechanisms of the stomach and intestinal mucosa. The development of peptic ulcers is influenced through a mixture of infectious, pharmacological, environmental, lifestyle, and host-related issues. Although gastric acid and pepsin are essential components in ulcer formation, they are not the sole causes of the disease. Various etiological factors weaken the mucosal resistance system, creation the gastrointestinal lining additional liable to injury.^[47]

1.5.1 *Helicobacter pylori* Infection

Peptic ulcer illness is thought to be significantly caused by *H. pylori*, a gram-negative, helical, microaerophilic bacterium that lives in the stomach mucosa. By producing the enzyme urease, which breaks down urea into ammonia and carbon dioxide, it is able to survive in the acidic environment of the stomach. Ammonia neutralises stomach acid in the bacterium's immediate environment, allowing it to survive in the protective mucus layer. Persistent colonization by *H. pylori* induces chronic inflammation of the gastric mucosa through the release of bacterial virulence factors and stimulation of the host immune response. The infection promotes infiltration of inflammatory cells, increases the creation of cytokines such as TNF α and IL6, and enhances oxidative stress. These changes damage epithelial cells, reduce mucus production, impair bicarbonate secretion, and disrupt the honor of the stomach mucosal barrier.^[48]

In addition to mucosal injury, *H. pylori* alters gastric acid secretion, depending on the site and severity of infection. Increased acid secretion may predispose individuals to duodenal ulcers, whereas chronic inflammation involving the gastric body may reduce acid secretion but weaken mucosal defense, contributing to gastric ulcer formation. Persistent infection also delays ulcer healing and increases the likelihood of recurrence if left untreated.^[49]

1.5.2 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs are among the leading causes of peptic ulcer disease, particularly in elderly individuals and patients requiring long-term pain management. Common NSAIDs include aspirin, ibuprofen, diclofenac,

naproxen, and indomethacin. These drugs exert their therapeutic properties through stop cyclooxygenase (COX) enzymes, thereby reducing prostaglandin synthesis. The stomach mucosa relies on prostaglandins to stay intact. They regulate inflammatory responses, keep blood flowing to the mucosa, help the epithelium heal, and increase mucus and bicarbonate output. These protective actions are compromised and acid-related damage is more likely to occur when nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin synthesis. NSAIDs also produce direct topical irritation of the gastric epithelium. They increase epithelial permeability, allowing hydrogen ions to diffuse into the mucosa, where they cause cellular damage and inflammation. Long-term NSAID therapy is therefore associated with gastric erosions, ulcer formation, gastrointestinal bleeding, and perforation. The risk of NSAID-induced ulceration increases with advancing age, prolonged treatment duration, higher drug doses, concurrent corticosteroid or anticoagulant therapy, previous past of peptic ulcer illness, and *H. pylori* infection.^[50]

1.5.3 Alcohol Consumption

Excessive alcohol intake is another important factor that contributes to gastric mucosal injury. Alcohol directly damages epithelial cells by disrupting membrane integrity, increasing mucosal permeability, and dissolving the defensive secretion coating. This facilitates the penetration of stomach acid and pepsin into deeper tissue layers, leading to inflammation and ulcer formation. Alcohol consumption also decreases gastric mucosal plasma movement, increases oxidativestress, promotes lipid peroxidation, and stimulates the making of inflammatory mediators. Responsive oxygen species generated during alcohol-induced wound damage cellular proteins, lipids, and DNA, resulting in epithelial necrosis and delayed tissue repair. Experimental ethanol-induced gastric ulcer models are widely used in pharmacological research because ethanol rapidly produces reproducible gastric lesions through oxidative stress, vascular injury, inflammation, and disruption of mucosal defense mechanisms.^[51]

1.5.4 Stress

Stress has an significant contributing issue in the development of pepticulcer disease, particularly in critically ill patients experiencing some physical stress like as trauma, burns, main surgery, sepsis, or shock. Stress-related gastric mucosal injury results primarily from reduced mucosal blood flow, ischemia, oxidative stress, and inflammatory activation. Activation of the hypothalamic–pituitary–adrenal axis during stress rises glucocorticoid secretion and sympathetic nervous system activity. These physiological changes reduce gastric perfusion, impair mucus production, and compromise epithelial repair mechanisms. Stress may also alter gastric motility and acid secretion, further increasing susceptibility to mucosal injury. Psychological stress

may indirectly contribute to ulcer development by promoting unhealthy lifestyle habits, including smoking, alcohol consumption, irregular eating patterns, and increased use of NSAIDs. Although psychological stress alone is rarely sufficient to cause peptic ulcers, it can aggravate existing gastric damage and delay ulcer healing.^[52]

1.5.5 Smoking

Cigarette smoking is a unshakable risk issue for peptic ulcer illness and its complications. Smoking reduces stomach mucosal plasma movement, decreases bicarbonate emission, inhibits prostaglandin synthesis, and impairs mucus production, thereby weakening the gastric mucosal barrier. Nicotine and other toxic compounds present in tobacco smoke increase oxidative stress and stimulate the release of inflammatory mediators. Smoking may also interfere with the healing of existing ulcers by delaying epithelial regeneration and reducing tissue oxygenation. Furthermore, smokers have a higher risk of ulcer recurrence and gastrointestinal bleeding than non-smokers. Several studies have demonstrated that smoking enhances the ulcerogenic properties of *H. pylori* infection and NSAID therapy, making ulcer disease more severe and more difficult to treat.^[53]

1.5.6 Dietary Factors

Dietary habits can influence gastric mucosal health, although diet alone is not considered a primary reason of peptic ulcer illness. Frequent consumption of highly spicy foods, excessive caffeine, carbonated beverages, and foods rich in salt or preservatives may irritate the gastric mucosa in susceptible individuals. Irregular meal timing and prolonged fasting may also increase gastric acid exposure and aggravate dyspeptic symptoms. Conversely, diets rich in fruits, vegetables, dietary fiber, and natural antioxidants may support gastric mucosal protection by reducing oxidative stress and inflammation. Polyphenols, flavonoids, vitamins C and E, carotenoids, and other naturally occurring phytochemicals possess antioxidant and cytoprotective properties that may help preserve mucosal integrity. Adequate nutrition also supports tissue repair, immune function, and maintenance of the gastric epithelial barrier. Therefore, healthy dietary practices may contribute to ulcer prevention and enhance recovery in patients receiving appropriate medical therapy.^[54]

1.6 Pathophysiology of Gastric Ulcer

Gastric ulcer is a multifactorial pathological condition described thru disruption of the stomach mucosal surface and formation of a localized lesion that extends beyond the superficial epithelial layer. The growth of a stomach ulcer is primarily associated with an difference between aggressive factors capable of damaging the gastric mucosa and defensive mechanisms responsible for maintaining mucosal integrity. Gastric hydrochloric acid and pepsin constitute important aggressive factors; however, their presence alone is insufficient to explain

ulcer formation because the healthy stomach is continuously exposed to these substances without developing ulceration. Ulcer formation occurs when mucosal defense, repair, and adaptation become inadequate in relation to the magnitude and duration of mucosal injury. Under normal physiological conditions, gastric epithelial cells are protected by a mucus-bicarbonate layer, intact epithelial tight junctions, acceptable mucosal plasma flow, endogenous prostaglandins, nitric oxide, antioxidant methods, and rapid epithelial restitution. These mechanisms act together to prevent hydrogen ions and other damaging substances from reaching deeper tissues. When one or more of these protective mechanisms are compromised, the permeability and resistance of the mucosa may change, allowing acid and pepsin to penetrate the damaged epithelial barrier.^[55]

Gastric acid is concealed thru parietal cells situated predominantly in the gastric glands of the fundus and body. Hydrochloric acid creates a highly acidic gastric environment that facilitates protein digestion and provides an important defense against ingested microorganisms. However, excessive acid exposure or impaired mucosal protection can contribute to ulcer development. Hydrogen ions can diffuse backward through damaged epithelial regions and enter the mucosa, producing intracellular acidification, mitochondrial dysfunction, cellular injury, and tissue necrosis. Pepsin also contributes to mucosal damage. It is derived from pepsinogen released by gastric chief cells and becomes activated under acidic conditions. Once activated, pepsin participates in protein digestion but may also aggravate mucosal injury when the epithelial barrier has been disrupted. Acid and pepsin can therefore act synergistically in the progression of an established mucosal lesion. Oxidative stress represents another important component of gastric ulcer pathogenesis. Reactive oxygen species can be generated during inflammation, ischemia-reperfusion, ethanol exposure, drug-induced injury, and other pathological conditions. Increased lipid peroxidation may further weaken the gastric epithelial barrier and delay mucosal recovery.^[56]

Inflammation is closely associated with oxidative injury during gastric ulcer formation. Excessive inflammation may also interfere with epithelial proliferation and tissue repair. Gastric microcirculation is another critical determinant of mucosal integrity. Adequate blood flow supplies oxygen and nutrients and facilitates removal of hydrogen ions and toxic metabolites. Reduced mucosal perfusion can produce tissue hypoxia and impair epithelial repair. Ischemia followed by restoration of blood flow may additionally increase oxidative stress over the peaks of responsive oxygen species. Consequently, disturbances in gastric microcirculation can contribute to both initiation and progression of ulcerative injury. Prostaglandins provide an important endogenous defense against gastric ulceration. They encourage saliva and bicarbonate emission, maintain

mucosal plasma movement, support epithelial restitution, and contribute to regulation of inflammatory responses. Suppression of prostaglandin synthesis, particularly through inhibition of cyclooxygenase enzymes by NSAIDs, can significantly weaken gastric mucosal defense and increase susceptibility to ulcer formation. The overall pathophysiology of gastric ulcer can therefore be viewed as a dynamic imbalance between aggressive and protective mechanisms. Factors such as *Helicobacter pylori**, NSAIDs, alcohol, smoking, physiological stress, and oxidative injury can disturb this equilibrium. The resulting impairment of mucosal defense, increased inflammatory activity, oxidative damage, reduced blood flow, and delayed epithelial repair may allow superficial mucosal damage to progress into a deeper ulcerative lesion.^[57]

1.7 Gastric Mucosal Defense Mechanisms

The gastric mucosa possesses an integrated defense system that allows it to withstand continuous exposure to hydrochloric acid, pepsin, food components, microorganisms, and potentially harmful chemical substances. Gastric mucosal protection is not dependent on a single mechanism but involves several complementary mechanisms, with the mucus-bicarbonate barrier, epithelial integrity, mucosal plasma movement, prostaglandins, nitric oxide, antioxidant defenses, and continuous epithelial renewal.^[58]

1.7.1 Mucus-Bicarbonate Barrier

The mucus-bicarbonate system represents one of the first lines of defense against gastric acid and pepsin. Surface mucous cells continuously secrete mucus that forms a viscous layer over the gastric epithelium. This layer acts as a physical wall and decreases direct contact between epithelial surface and luminal contents. Bicarbonate secreted by epithelial cells becomes trapped within the mucus layer and helps maintain a relatively less acidic microenvironment close to the epithelial surface. Although the gastric lumen may contain highly acidic contents, the pH immediately adjacent to healthy epithelial cells is maintained at a considerably higher level. Disruption of the mucus-bicarbonate barrier can increase hydrogen-ion penetration and make the mucosa more susceptible to injury.^[59]

1.7.2 Gastric Epithelial Barrier

The epithelial layer provides another important component of gastric mucosal defense. Gastric epithelial cells are closely connected through specialized junctional structures, including tight junctions, which restrict the movement of hydrogen ions and other potentially harmful substances through the intercellular spaces. The integrity of epithelial cell membranes and junctional complexes is essential for maintaining barrier function. When epithelial cells are damaged by ethanol, NSAIDs, oxidative stress, or other agents, permeability may increase and hydrogen ions can move into deeper tissues. Rapid restoration of epithelial continuity is therefore

essential for preventing progression of superficial injury.^[60]

1.7.3 Mucosal Blood Flow

Passable gastric mucosal plasma movement was essential for maintaining tissue integrity. The gastric circulation supplies oxygen and nutrients required for cellular metabolism and tissue repair. It also facilitates the removal of hydrogen ions and potentially toxic metabolic products that accumulate following mucosal injury. Adequate perfusion supports the synthesis of mucus and bicarbonate and contributes to epithelial restitution. Reduced mucosal blood flow can cause hypoxia, energy depletion, and increased susceptibility to acid-mediated damage. Ischemia-reperfusion injury can further increase reactive oxygen species generation and amplify mucosal damage.^[61]

1.7.4 Prostaglandin-Mediated Protection

Endogenous prostaglandins, particularly those derived from cyclooxygenase pathways, are important regulators of gastric mucosal protection. They stimulate mucus and bicarbonate emission, support mucosal plasma movement, promote epithelial compensation, and help regulate inflammatory processes. The importance of prostaglandins is particularly evident in NSAID-induced gastric injury. Inhibition of cyclooxygenase activity reduces prostaglandin synthesis and consequently weakens several protective mechanisms simultaneously. This provides an important mechanistic explanation for the ulcerogenic effects of prolonged NSAID exposure.^[62]

1.7.5 Nitric Oxide

Nitric oxide (NO) was an endogenous waving particle intricate in regulation of stomach mucosal plasma movement and mucosal defense. It contributes to vascular relaxation and maintenance of adequate microcirculation. NO may also influence mucus secretion, epithelial integrity, inflammatory responses, and tissue repair. Appropriate nitric oxide signaling can therefore support gastric mucosal resistance against injury. Disturbance of NO-related pathways under pathological conditions may contribute to impaired blood flow and increased mucosal susceptibility.^[63]

1.7.6 Antioxidant Defense System

Gastric tissues possess endogenous antioxidant mechanisms that protect cells from oxidative injury. Significant enzymatic antioxidants contain SOD, catalase, and glutathione-dependent enzymes. Reduced glutathione (GSH) and other non-enzymatic antioxidants also contribute to maintenance of cellular redox balance. SOD converts superoxide extremists into hydrogenperoxide, though catalase and other enzymes help change hydrogenperoxide into less harmful products. When reactive oxygen species production becomes excessive, these systems may become overwhelmed, resulting in lipid peroxidation, protein oxidation, mitochondrial injury, and cellular dysfunction. Therefore, preservation or enhancement of endogenous

antioxidant defenses may contribute significantly to gastroprotection. This mechanism is particularly relevant when investigating medicinal plants containing phenolic compounds, flavonoids, and other antioxidant phytoconstituents.^[64]

1.7.7 Epithelial Restitution and Cell Renewal

The gastric epithelium possesses a remarkable ability to repair superficial damage. Following epithelial injury, viable cells surrounding the damaged region migrate into the injured area and restore epithelial continuity. This rapid process is known as epithelial restitution. Long-term maintenance of mucosal integrity additionally depends on epithelial cell proliferation and differentiation. Continuous renewal replaces damaged or senescent cells and preserves the functional gastric barrier. Factors that impair cell proliferation or tissue regeneration may consequently delay ulcer healing.^[65]

1.7.8 Immune and Inflammatory Regulation

The gastric mucosa contains immune cells and signaling systems that help maintain tissue homeostasis and respond to infection or injury. Controlled inflammatory responses contribute to defense and tissue repair, whereas excessive or persistent inflammation may promote mucosal injury. Regulation of inflammatory mediators such as TNF α , IL1 β , and IL6 is therefore important in maintaining gastric mucosal integrity. Excessive activation of inflammatory pathways can increase oxidative stress and interfere with epithelial regeneration.^[66]

1.7.9 Integrated Gastric Mucosal Defense

Gastric mucosal defense is consequently the result of coordinated activity among mucus and bicarbonate secretion, epithelial integrity, adequate blood circulation, prostaglandins, nitric oxide, antioxidant systems, and cellular renewal. Failure of one mechanism may be partially compensated by others; however, simultaneous impairment of multiple defenses can markedly increase susceptibility to ulcer formation. For the investigation of potential gastroprotective agents, evaluation of multiple components of this defense system can provide more meaningful evidence than assessment of gastric acidity alone. Changes in gastric pH, mucus protection, antioxidant enzymes, oxidative stress markers, inflammatory mediators, and histological architecture can collectively help explain the mechanism underlying anti-ulcer activity.^[67]

1.8 Gastric Acid Secretion and Regulation

Gastric hydrochloric acid is an essential physiological secretion involved in protein digestion, activation of pepsin, and defense against ingested microorganisms. Acid is secreted primarily by parietal cells situated within the oxyntic glands of the stomach fundus and body. Although gastric acid is necessary for normal digestive function, uncontrolled secretion or exposure of a compromised mucosa to acid can contribute to the development and persistence of gastric ulceration.^[68]

1.8.1 Gastric Acid Secretion through Parietal Cells

The final step in hydrochloric acid emission occurs by the activity of gastric $H^+/K^+ATPase$, usually mentioned to as the protonpump, and situated on the secretory canalicular membrane of parietal cells. This transporter exchanges intracellular hydrogen ions for extracellular potassium ions and thereby contributes to the emission of hydrogenions into the gastric lumen. With the help of carbonic anhydrase, carbon dioxide and water are able to mix within the parietal cell to form carbonic acid. Hydrogen ions and bicarbonate are the byproducts of carbonic acid's subsequent separation. Through the $H^+/K^+-ATPase$, hydrogen ions are transported into the stomach lumen, and bicarbonate is released into the circulation in return for chloride ions. Hydrochloric acid is produced when chloride combines with hydrogen ions after entering the stomach lumen through chloride channels.^[69]

The overall process can be represented as
 $CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$

The generated H^+ is secreted through the $H^+/K^+-ATPase$, while Cl^- enters the gastric lumen and combines with hydrogen ions to form HCl .

1.8.2 Major Regulators of Gastric Acid Secretion

Gastric acid emission is controlled through neural, hormonal, and paracrine mechanisms. The three major stimulatory pathways involve acetylcholine, gastrin, and histamine.^[70]

1.8.2.1 Acetylcholine

Acetylcholine was free from vagal parasympathetic nerve finales and stimulates acid secretion primarily through muscarinic receptors on parietal cells. Vagal stimulation also promotes gastric acid secretion indirectly by stimulating gastrin release and influencing histamine-mediated signaling.

The vagal pathway is particularly important during the cephalic and gastric phases of digestion.

1.8.2.2 Gastrin

Gastrin has a peptide hormone produced predominantly through G cells in the stomach antrum. Its issue is enthused through the occurrence of food, particularly peptides and aminoacids, as well as by neural signals.

Gastrin promotes gastric acid secretion directly and indirectly. It stimulates parietal cells and, importantly, promotes histamine issue from enterochromaffin-like cells. Gastrin therefore amplifies acid secretion through interaction with other regulatory pathways.

1.8.2.3 Histamine

Histamine acts synergistically with acetylcholine and gastrin, producing a strong stimulatory effect on the parietal cell. This interaction explains why inhibition of H_2 receptors or the proton pump can substantially reduce gastric acid secretion.

1.8.2.4 Gastric Phase

The gastric phase begins when food pass in the abdominal. Distension of the stomach starts mechanoreceptors and vagal reflexes, while peptides and amino acids stimulate gastrin secretion. Gastrin and histamine subsequently promote parietal cell activity and increase hydrochloric acid secretion.

1.8.2.5 Intestinal Phase

The duodenal phase activates when gastric contents enter the duodenum. Originally, intestinal components may produce a modest stimulatory effect on gastric secretion. However, as acidic chyme and nutrients enter the small intestine, inhibitory mechanisms become increasingly important. Neural and hormonal signals from the intestine reduce gastric acid secretion and slow gastric emptying, thereby preventing excessive acid delivery to the duodenum.

1.8.2.6 Inhibitory Regulation of Gastric Acid Secretion

Gastric acid emission is controlled by several negative feedback mechanisms. Increasing acidity within the gastric antrum promotes the release of somatostatin from D cells. Somatostatin inhibits gastrin release and thereby reduces stimulation of parietal cells.

The entry of acidic gastric contents into the duodenum also activates intestinal inhibitory pathways. Secretin and other intestinal mediators contribute to reduction of gastric acid secretion and promote bicarbonate secretion, helping to neutralize gastric acid entering the small intestine.

1.8.2.7 Association between Acid Secretion and Gastric Ulceration

Gastric acid has a central role in the progression of ulcerative lesions because hydrogen ions can penetrate damaged mucosa and aggravate epithelial injury. However, the relationship between acid secretion and ulcer formation is more complex than simple acid overproduction. Many patients with gastric ulcers do not necessarily have excessive basal acid secretion. Instead, impaired mucosal defense can make normal acid concentrations damaging to the gastric tissue.

This concept is particularly important in drug-induced and ethanol-induced ulceration. NSAIDs can reduce prostaglandin-mediated defense, while ethanol can disrupt epithelial integrity and increase oxidative stress. In both situations, gastric acid may worsen the resulting mucosal damage even when acid production itself is not markedly elevated.

Consequently, effective gastroprotection may involve both reduction of excessive acid-related injury and strengthening of endogenous mucosal defense. Evaluation of gastric acid secretion, gastric pH, mucus production, oxidative stress, antioxidant defenses, inflammatory mediators, and histological changes can

provide a comprehensive understanding of the anti-ulcer activity of a test substance such as the ethanolic leaf extract of *Torenia crustacea*.

1.9 Role of Mucus, Bicarbonate and Prostaglandins

Gastric mucus forms a defensive layer over the abdominal lining and limits direct contact of epithelial cells with hydrochloric acid and pepsin. Bicarbonate trapped within the mucus helps neutralize hydrogen ions and maintains a relatively favorable pH near the epithelial surface.^[71]

1.10 Oxidative Stress and Gastric Ulceration

Oxidative stress plays an important role in gastric mucosal injury. Extreme construction of reactive oxygen classes can cause lipid peroxidation, protein harm, mitochondrial dysfunction, and epithelial injury. During ulceration, endogenous antioxidant defenses like as superoxide, and reduced glutathione may become depleted, while lipid peroxidation products such as malondialdehyde may increase. Therefore, antioxidant activity is an important mechanism through which potential gastroprotective agents may reduce gastric injury.^[72]

1.11 Inflammatory Mediators Involved in Ulcerogenesis

Inflammation contributes significantly to gastric ulcer formation and progression. Gastric injury can stimulate inflammatory mediators like as TNF α , IL1 β , and IL6 and activate gesturing paths with NF- κ B. Excessive swelling activation can increase oxidative damage, disrupt epithelial integrity, and delay mucosal healing. Regulation of these inflammatory responses may therefore contribute to gastroprotection.^[73]

1.12 Current Treatment of Peptic Ulcer

Proton-pump inhibitors like as omeprazole and pantoprazole are broadly used for acid suppression, while H₂-receptor antagonists and antacids may provide additional acid control or symptomatic relief. Sucralfate and bismuth preparations provide mucosal protection. In *H. pylori*-associated ulcers, eradication therapy using appropriate antimicrobial combinations with acid suppression is essential. NSAID-associated ulcers may require withdrawal or modification of the offending drug along with gastroprotective therapy.^[74]

1.13 Limitations of Conventional Anti-Ulcer Therapy

Although conservative anti-ulcer are effective, recurrence, adverse effects, drug interactions, antimicrobial resistance, treatment cost, and prolonged medication requirements remain important concerns. Acid suppression primarily addresses gastric acidity and may not directly correct oxidative stress, inflammation, or other abnormalities involved in mucosal injury. These limitations have encouraged research into alternative and complementary gastroprotective agents.^[75]

1.14 Role of Medicinal Plants in Anti-Ulcer Therapy

Therapeutic floras are progressively investigated as possible bases of gastroprotective mediators because they comprise diverse phytoconstituents such as flavonoids, phenolics, tannins, terpenoids, and alkaloids. These compounds may provide antioxidant, anti-inflammatory, antimicrobial, cytoprotective, and mucosal-protective effects. Plant extracts may therefore act through multiple mechanisms, including reduction of oxidative stress, suppression of inflammation, enhancement of mucus production, and preservation of gastric mucosal integrity. Scientific evaluation of such plants can help identify novel natural sources of anti-ulcer agents.^[76]

1.15 Rationale for Selecting *Torenia crustacea*

The selection of *Torenia crustacea* for the present investigation is based on the increasing interest in therapeutic floras as potential bases of safe and biologically active therapeutic agents. Plant-derived phytoconstituents such as flavonoids, phenolic compounds, tannins, and terpenoids had associated with antioxidant, anti-inflammatory, antimicrobial, and tissue-protective activities, which are relevant to the prevention and management of gastric mucosal injury. Gastric ulceration is a multifactorial process involving oxidative stress, inflammation, disruption of the mucus-bicarbonate barrier, impaired mucosal defense, and epithelial damage. Therefore, a plant possessing antioxidant and anti-inflammatory potential may provide gastroprotective effects through multiple complementary mechanisms. *Torenia crustacea* was selected as a candidate plant for investigating these possibilities using its ethanolic leaf extract. The current investigation is intended to assess the antiulcer possible of the ethanolic extract of *Torenia crustacea* leaves through appropriate experimental models and biochemical and histopathological parameters. The findings may provide preliminary scientific evidence regarding its gastroprotective potential and establish a basis for further phytochemical and mechanistic investigations. Thus, the selection of *Torenia crustacea* is justified by the potential biological relevance of its phytoconstituents, the need to investigate additional plant-derived gastroprotective candidates, and the possibility of identifying a natural source with anti-ulcer activity.^[77]

1.16 Plant Profile

Plant name: *Torenia crustacea*

Botanical Name

Torenia crustacea (L.) Cham. & Schltdl.

Common Names

The plant is commonly known by regional or vernacular names, which may vary according to geographical location. The locally used name should be reported according to the area from which the plant material is collected.^[78]



Figure 1.1: Leaves of *Torenia crustacea*.

Botanical Classification

Kingdom: Plantae
Clade: Angiosperms
Order: Lamiales
Family: Linderniaceae
Genus: *Torenia*
Species: *Torenia crustacea* (L.) Cham. & Schltdl.

Morphological Characteristics

Root

The plant possesses a relatively delicate root system that anchors the herb in the soil and facilitates absorption of water and minerals.

Stem

The stem is generally slender and herbaceous, with branching that contributes to the spreading or low-growing habit of the plant. The surface characteristics may vary depending on the developmental stage and growing conditions.

Leaves

The leaves are simple and green and are arranged along the stem. Leaf shape, margin, surface characteristics, and dimensions should be recorded from authenticated plant material collected for the present investigation.

Flowers

The flowers are relatively small and characteristic of the genus *Torenia*. Floral morphology, including colour, arrangement, calyx, corolla, stamens, and other diagnostic characters, should preferably be confirmed using authenticated botanical material.

Fruits and Seeds

Following flowering, the plant produces small fruits containing numerous seeds. Fruit and seed characteristics may be useful for botanical identification and authentication.^[79]

9. Geographical Distribution

Torenia crustacea has been reported from tropical and subtropical regions of Asia and other suitable warm and humid environments. Its occurrence may be associated with moist soils and disturbed or open habitats. The exact geographical distribution should be documented according to the authenticated specimen and collection site used in the present study.^[80]

Traditional and Ethnomedicinal Importance

Plants belonging to the genus *Torenia* have attracted interest because of their traditional medicinal relevance and reported biological activities. However, ethnomedicinal claims should be differentiated from experimentally established pharmacological effects. For the present study, any traditional use attributed specifically to *Torenia crustacea* should be supported by appropriate ethnobotanical literature.

Reported Phytochemical Constituents of *Torenia crustacea*

Torenia crustacea holds a varied variety of subordinate metabolites that may donate to its biological possessions. Tannins may contribute to astringent and mucosal-protective effects, whereas terpenoids and alkaloids may contribute to various pharmacological activities. Saponins and glycosides may also participate in the biological activity of the plant through different cellular and membrane-associated mechanisms.

For the present investigation, the ethanolic leaf extract of *Torenia crustacea* may be exposed to preliminary phytochemical showing for the qualitative detection of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, glycosides, and steroids. Further chromatographic and spectrometric investigations, such as HPTLC, HPLC, LC-MS, GC-MS, FTIR, or NMR, may be employed to establish the individual chemical constituents and provide a more definitive phytochemical profile. The exact chemical composition may vary according to geographical origin, plant part, environmental conditions, maturity, extraction method, and solvent used.

The presence of antioxidant and potentially anti-inflammatory phytochemical groups provides a rational basis for investigating the gastroprotective potential of *Torenia crustacea*.^[81]

Reported Pharmacological Potential

Plant-derived phenolic and flavonoid mixtures are frequently related through antioxidant and anti-inflammatory activities. These properties are relevant to gastrointestinal protection because oxidative stress and inflammation are important mechanisms involved in gastric mucosal injury. For this reason, *Torenia crustacea* may be considered a candidate for pharmacological investigation. However, the specific antiulcer activity of the ethanolic leaf extract should be established experimentally rather than inferred solely from phytochemical composition or traditional use.^[82]

Reason of the Study

Gastric ulcer remains a common gastrointestinal disorder associated with mucosal damage, oxidative stress, and inflammation. Although several anti-ulcer drugs are available, their prolonged use may be associated with adverse effects and recurrence. Therefore, there is a continuing need to explore safer plant-based alternatives. *Torenia crustacea* contains bioactive phytoconstituents with potential antioxidant and protective properties, but its anti-ulcer activity has not been sufficiently investigated. Hence, this study was undertaken to assess the gastro protective potential of the ethanolic extract of *Torenia crustacea* leaves using suitable experimental models.

Chapter 2

Review of Literature

Ghori et al. (2021) investigated the phytochemical and pharmacological properties of *Lindernia crustacea*, a plant taxonomically relevant to *Torenia crustacea*. The study focused on phytochemical investigation and LC-MS analysis of plant extracts. Different extracts were evaluated for their antibacterial and anthelmintic activities. The investigation demonstrated the occurrence of several lessons of secondary metabolites. The study highlighted the chemical diversity of the plant and its potential biological activities. LC-MS-based analysis provided additional information regarding the chemical

constituents present in the extract. The authors suggested that the observed biological effects might be related by the occurrence of bioactive phytochemicals. Such findings are relevant to the pharmacological exploration of *Torenia/Lindernia crustacea*. However, the study did not specifically investigate gastric ulcer protection. Therefore, its findings provide supportive phytochemical evidence rather than direct evidence of anti-ulcer activity.^[83]

Nath et al. (2024) investigated the phytochemical composition and invitro biological actions of *Torenia crustacea*. The researchers evaluated extracts of the plant for their phytochemical characteristics and antioxidant properties. The study demonstrated the presence of several biologically relevant phytochemical groups. Antioxidant evaluation indicated that the plant possesses free-radical-scavenging potential. The authors also investigated the anticancer activity of the extracts using appropriate cell-based models. Since oxidative stress is an important mechanism involved in gastric ulcerogenesis, the antioxidant findings are particularly relevant to gastroprotective research. However, anti-ulcer activity was not directly evaluated. Thus, this study provides a scientific rationale for investigating *Torenia crustacea* in experimental gastric ulcer models.^[84]

Prayoga et al. (2024) reviewed the anti-ulcer activity of medicinal plants using evidence from preclinical and clinical investigations. The authors examined studies involving different types of ulcer disorders, with particular emphasis on plant-derived therapeutic agents. The review described several mechanisms through which medicinal plants may protect gastric tissue. These mechanisms included enhancement of mucosal defense, reduction of oxidative stress, inhibition of inflammatory responses, and modulation of gastric acid secretion. The authors also highlighted the importance of active plant metabolites in gastroprotection. Several studies indicated that natural extracts can reduce ulcer formation and improve mucosal healing. The review further emphasized the need for appropriate quality control and standardized investigations of herbal preparations. The authors suggested that medicinal plants may provide useful leads for alternative gastroprotective agents. These findings support investigation of less-explored medicinal plants such as *Torenia crustacea*.^[85]

Keny and Kale (2022) reviewed medicinal plants with potential anti-ulcerogenic properties and discussed their possible mechanisms of action. The authors emphasized that gastric ulceration results from an imbalance between aggressive and protective factors. Their review considered several medicinal plants and their phytoconstituents in relation to gastroprotective effects. Antioxidant, anti-inflammatory, cytoprotective, and acid-regulating activities were identified as important mechanisms. The authors also discussed the relevance of flavonoids and other plant-derived constituents in reducing gastric mucosal injury. They highlighted

limitations associated with conventional anti-ulcer medicines, including recurrence and adverse effects. The review suggested that medicinal herbs may provide alternative sources of gastroprotective compounds. However, the authors also noted the need for further toxicity and mechanistic investigations. These observations provide a useful theoretical basis for evaluating *Torenia crustacea*.^[86]

Deshpande et al. (2024) reviewed the anti-ulcer potential of selected medicinal plants and the experimental models used for evaluating gastroprotective activity. The authors discussed different approaches for assessing gastric ulcer protection in laboratory animals. Ethanol-induced, NSAID-induced, and other experimental ulcer models were considered useful for screening plant extracts. The review highlighted the importance of determining ulcer index and percentage protection. It also emphasized biochemical parameters related to oxidative stress and antioxidant defense. According to the review, plant extracts may protect the gastric mucosa through multiple mechanisms rather than only reducing acid secretion. Phytoconstituents such as flavonoids, tannins, phenolics, and terpenoids were considered potentially important. The authors suggested that histopathological evaluation provides additional evidence of mucosal protection. These observations are directly relevant to the proposed evaluation of ethanolic *Torenia crustacea* leaf extract.^[87]

Ghori et al. (2021) performed a detailed phytochemical investigation of *Lindernia crustacea* using different extraction approaches. The study included LC-MS analysis to characterize potentially bioactive compounds. The authors also evaluated antibacterial activity against selected organisms. Anthelmintic action of the methanolic leaf extract had also been investigated. The results demonstrated that the plant possesses several phytochemical constituents with measurable biological activity. The findings support the traditional medicinal relevance of the species. The study is particularly important because it provides experimentally generated phytochemical evidence for *Lindernia crustacea*. Although gastric protection was not examined, the presence of biologically active constituents provides a rationale for further pharmacological studies. Such phytochemical information can support the selection of ethanol as an extraction solvent for subsequent investigations. Therefore, the study contributes indirectly to the rationale for exploring anti-ulcer activity of *Torenia crustacea*.^[88]

Nath et al. (2024) assessed the antioxidant potential of *Torenia crustacea* as part of a broader investigation of its biological properties. The study demonstrated that extracts of the plant possess measurable antioxidant activity. The authors associated this action with the occurrence of various secondary metabolites. Phenolic and flavonoid-type constituents are particularly relevant because of their ability to interact with reactive oxygen

species. Oxidative stress is known to contribute substantially to gastric epithelial injury. Excessive reactive oxygen species can promote lipid peroxidation, membrane damage, and disruption of mucosal integrity. Therefore, antioxidant activity observed in *Torenia crustacea* may have relevance to gastroprotective research. Nevertheless, the study did not establish anti-ulcer efficacy in an experimental gastric ulcer model. Further in-vivo investigations are therefore required to determine whether the antioxidant potential translates into gastric mucosal protection.^[89]

Prayoga et al. (2024) reported that medicinal plants can influence several mechanisms involved in ulcer development. Their review described protection of the gastric mucosa as one of the major mechanisms of plant-derived anti-ulcer activity. Reduction of inflammatory responses was also identified as an important therapeutic mechanism. Plant extracts may additionally decrease oxidative damage by enhancing endogenous antioxidant defenses. Some natural products can influence gastric acid secretion and reduce acid-mediated tissue injury. Other mechanisms include enhancement of mucus production and improvement of epithelial repair. The authors emphasized that multiple mechanisms may act simultaneously within a plant extract. Such multi-target effects may be advantageous in a multifactorial disorder such as peptic ulcer disease. The review supports further pharmacological evaluation of medicinal plants with limited experimental evidence. This framework is applicable to the investigation of *Torenia crustacea* leaf extract.^[90]

Keny and Kale (2022) discussed the relationship between phytochemical composition and anti-ulcer activity of medicinal herbs. These compounds can potentially reduce oxidative stress and support gastric mucosal integrity. Some phytochemicals may also influence inflammatory pathways involved in ulcer progression. Enhancement of mucus secretion and preservation of mucosal architecture were discussed as additional protective mechanisms. The authors highlighted that natural products may act through several pathways simultaneously. However, pharmacological activity should be confirmed using appropriate experimental models rather than being inferred solely from phytochemical screening. Toxicity and dose-related effects also require systematic assessment. This review provides useful mechanistic support for investigating plant-derived anti-ulcer agents. It also strengthens the rationale for studying the relatively less-explored *Torenia crustacea*.^[91]

Nath et al. (2024) reported that *Torenia crustacea* contains diverse phytochemical constituents that may contribute to its biological effects. The authors investigated the plant using phytochemical analysis followed by biological evaluation. Antioxidant activity was assessed to determine the ability of the extract to counter oxidative stress. The study further evaluated

anticancer effects in appropriate in-vitro models. The observed biological activities demonstrated that the plant possesses pharmacologically relevant constituents. The authors emphasized the importance of naturally occurring compounds in the development of potential therapeutic agents. These findings are particularly relevant to disorders in which oxidative stress contributes to tissue damage. Gastric ulceration represents one such condition in which oxidative injury can impair mucosal defense. Therefore, the findings provide supportive evidence for further gastroprotective investigation. Direct anti-ulcer efficacy, however, remains to be established experimentally.^[92]

Ghori et al. (2021) investigated the biological properties of *Lindernia crustacea* using different plant extracts. Their investigation included phytochemical profiling and LC-MS-based chemical characterization. Antibacterial activity was evaluated against selected microorganisms. The authors also assessed anthelmintic activity using the methanolic leaf extract. The study demonstrated that the plant contains chemically diverse constituents associated with biological effects. The findings support the ethnomedicinal importance of the plant. The absence of direct anti-ulcer investigation represents an opportunity for further research. Therefore, evaluation of ethanolic leaf extract in established gastric ulcer models may expand the pharmacological profile of the plant.^[93]

Prayoga et al. (2024) summarized experimental evidence showing that plant-derived products may reduce ulcer severity and promote mucosal recovery. Their review emphasized that gastroprotection can involve reduction of gastric lesions, suppression of inflammatory mediators, and improvement of mucosal integrity. Antioxidant effects were also repeatedly identified as an important component of plant-mediated protection. They also emphasized the need for appropriate experimental validation of herbal medicines. Standardization of plant material and extracts was identified as an important requirement for reproducible pharmacological findings. The review suggested that plant-derived therapies could complement existing approaches to ulcer management. However, more rigorous clinical and mechanistic evidence remains necessary. These observations provide a strong background for studying novel medicinal plants such as *Torenia crustacea*.^[94]

Deshpande et al. (2024) discussed experimental approaches used to evaluate anti-ulcer activity of medicinal plants. The authors described ethanol-induced gastric ulcer as an important model for investigating mucosal damage and gastroprotection. Experimental models can provide information regarding ulcer index, gastric secretion, mucus protection, and biochemical changes. Oxidative stress markers such as lipid peroxidation and antioxidant enzymes can help explain the underlying mechanism. Histopathological examination can additionally confirm epithelial

disruption, edema, inflammation, and restoration of gastric architecture. The review emphasized that combining macroscopic, biochemical, and histological parameters provides more reliable evidence. Plant extracts showing activity across these parameters may possess multifactorial gastroprotective effects. This experimental framework can be applied to ethanolic *Torenia crustacea* leaf extract. Such an approach may help determine whether the extract protects gastric tissue through antioxidant, anti-inflammatory, or mucosal mechanisms.^[95]

Nath et al. (2024) described *Torenia crustacea* as an ethnomedicinal plant used traditionally in regions including India, Indonesia, and Malaysia. Traditional applications include several inflammatory and skin-related conditions as well as other ailments. The authors investigated the plant scientifically to evaluate its phytochemical profile and biological properties. Their work demonstrated measurable antioxidant activity in plant extracts. The study also investigated in-vitro anticancer potential, thereby expanding the pharmacological evidence available for the species. The authors emphasized the importance of investigating lesser-studied medicinal plants for new bioactive compounds. Traditional use alone cannot establish efficacy for gastric ulcer disease, but it provides a basis for pharmacological exploration. The antioxidant properties observed in the study are relevant because oxidative stress is involved in gastric mucosal injury. Consequently, further evaluation of *Torenia crustacea* in anti-ulcer models is scientifically justified.^[96]

Keny and Kale (2022) highlighted the need for alternative approaches to the management of gastric ulceration. According to their review, conventional anti-ulcer drugs can be associated with recurrence, adverse effects, cost, and drug-interaction concerns. Medicinal plants have traditionally been used for gastrointestinal disorders and may contain multiple bioactive compounds. The authors discussed evidence supporting antioxidant, anti-inflammatory, cytoprotective, and mucosal-protective mechanisms. They also emphasized that the phytochemical composition of a medicinal plant can provide clues regarding its possible pharmacological activity. However, toxicity, standardization, and mechanistic validation remain necessary before therapeutic application. The review supports systematic investigation of less-characterized medicinal plants. In this context, *Torenia crustacea* represents an interesting candidate because its pharmacological profile is still comparatively underexplored. Therefore, evaluating its ethanolic leaf extract in experimental gastric ulcer models may address an important research gap.^[97]

AIM AND OBJECTIVE

Aim: To evaluate the anti-ulcer activity of the ethanolic extract of *Torenia crustacea* leaves using suitable experimental models.

OBJECTIVE

1. To collect, authenticate, and prepare the ethanolic leaf extract of *Torenia crustacea* using the maceration method.
2. To classify the main phytochemical constituents current in the ethanolic leaf extract of *Torenia crustacea* through preliminary phytochemical screening and thin-layer chromatography (TLC).
3. To evaluate the acute oral toxicity and safety profile of the ethanolic leaf extract of *Torenia crustacea* in experimental animals.
4. To investigate the anti-ulcer action of the ethanolic leaf extract of *Torenia crustacea* against ethanol-induced gastric ulceration in Wistar rats at different dose levels.
5. To assess the gastroprotective potential of the extract by evaluating gastric ulcer indices, oxidative stress biomarkers (MDA, SOD, CAT, and GSH), and histopathological changes in gastric tissue.

Plan of Work

1. Collection and Authentication of Plant Material
2. Preparation of Plant Extract
3. Thin-Layer Chromatography (TLC)
4. Preliminary Phytochemical Screening
5. Acute Oral Toxicity Study
6. Evaluation of Anti-Ulcer Activity
7. Assessment of Gastric Ulceration
8. Estimation of Oxidative Stress Markers
9. Histopathological Examination
10. Statistical Analysis and Interpretation

Chapter 3**MATERIALS AND METHODS****3.1 Collection of Plant Material**

Fresh leaves of *Torenia crustacea* had been collected from the campus premises of **Chaudhary Charan Singh University (CCS University), Meerut, Uttar Pradesh, India**. The plant material was collected during the appropriate season and carefully selected to obtain healthy and disease-free leaves. The collected leaves were thoroughly examined to remove damaged, infected, or extraneous plant materials. After collection, the leaves were washed gently with clean water to remove adhering soil, dust, and other impurities. The plant material was subsequently subjected to shade drying under suitable environmental conditions to minimize degradation of thermolabile and photosensitive constituents. The dried leaves were then powdered using a suitable mechanical

grinder and stored in a clean, dry, airtight container until further extraction and pharmacological investigation.

3.2 Authentication of Plant Material

The collected leaves of *Torenia crustacea* were authenticated by **Dr. Professor Vijay Malik, Chaudhary Charan Singh University (CCS University), Meerut, Uttar Pradesh, India**, based on their morphological and botanical characteristics. The plant material was identified as *Torenia crustacea* (L.) Cham. & Schltdl., belonging to the family **Linderniaceae**. The authenticated plant material was subsequently used for further extraction and pharmacological investigations. A voucher specimen of the herb material had preserved for upcoming reference.

3.3 Preparation of Ethanolic Extract of *Torenia crustacea* Leaves

The authenticated leaves of *Torenia crustacea* were collected and carefully washed with water to eliminate observing dirt and other peripheral materials. The leaves had shadedried at room temperature under well-ventilated conditions until a constant mass was obtained. The dried leaves were then coarsely powdered with a suitable automatic mill and kept into an airtight container until extraction.

The powdered plant material had been exposed to extraction with ethanol as the extracting solvent by the maceration method. A measured quantity of the powdered leaves was transferred into a clean, dry, closed container and sufficient quantity of ethanol was added to completely immerse the plant material. The mixture was kept for an appropriate period with intermittent stirring or shaking to facilitate penetration of the solvent and extraction of soluble phytoconstituents.

After the extraction was finished, the mixture was filtered through the right kind of paper to separate the marc from the liquid extract. The collected filtrate was reduced in temperature-sensitive components by concentrating it at reduced pressure using a rotary evaporator operating at a controlled temperature. A semisolid or dry ethanolic extract was obtained by further drying the concentrated extract.^[98]

The obtained ethanolic extract was weighed, and the percentage yield was calculated using the following formula:

$$\text{Percentage yield (\%)} = (\text{Weight of dried extract} / \text{Weight of powdered plant material}) \times 100$$

The prepared ethanolic abstract had moved into a clean, sealed vessel and kept under suitable conditions till further phytochemical screening and pharmacological evaluation.

3.4 TLC

The ethanolic leaf extract of *Torenia crustacea* was dissolved in methanol and applied as small spots on pre-

coated silica gel 60 F254 TLC plates. The plate had developed in a suitable optimized mobile phase inside a saturated TLC chamber. After development, the plate was removed, dried, and examined under UV light at 254 nm. The detached spots were recorded, and their R_f values had been calculated using.^[99]

$$Rf = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent front}}$$

3.5 Phytochemical Screening^[100]

1. Test for Alkaloids

Mayer's Test: A some droplets of Mayer's reagent had been mixed to the filtrate. The appearance of a cream or pale precipitate indicated the possible occurrence of alkaloids.

2. Test for Flavonoids

Shinoda test: Approximately 100 mg of extract was dissolved in a minor volume of ethanol. A few fragments of magnesium were added, followed by dilute hydrochloric acid. Development of a pink, reddish or orange coloration suggested the presence of flavonoid compounds.

3. Test for Phenolic Compounds

100mg of extract had mixed into distilled water or a suitable solvent. A some droplets of ferric chloride solution had been mixed. Growth of a blue, green, violet or dark coloring suggested the occurrence of phenolic constituents.

4. Test for Tannins

A small quantity of extract had been mixed in purified water and filtered. A some droplets of ferricchloride solution had mixed to the filtrate. Creation of a blueblack coloration shown the possible occurrence of tannins.

5. Test for Saponins

The extract had mixed in water and vigorously shaken in a test tube. Persistent development of a constant froth or foam showed the possible occurrence of saponins.

6. Test for Terpenoids

Approximately 100 mg of extract had mixed with 10mL of purified water and vigorously shaken in a graduated test tube. The development of a determined froth layer that persisted for numerous minutes suggested the occurrence of saponins.

7. Test for Glycosides

The extract had been subjected to an appropriate glycoside detection test based on hydrolysis and subsequent chemical reaction. Development of the characteristic colour reaction indicated the possible presence of glycosidic constituents.

8. Test for Steroids

The extract had eact through chloroform shadowed thru acetic anhydride and concentrated sulfuricacid. Development of a characteristic colour change, such as green or bluish-green, was measured revealing of steroidal constituents.

9. Test for Amino Acids

The aqueous extract solution had reacting with a some droplets of **ninhydrin reagent** and heated gently. Expansion of a **blue or purple coloration** specified the possible occurrence of free amino acids.

10. Test for Carbohydrates

Filtered water was used to dissolve a little amount of the extract. After the filtrate was treated with Molisch's reagent, powerful sulphuric acid was carefully added along the test tube's edge. The presence of carbohydrates was indicated by the appearance of a ring of violet or purple at the boundary.

11. Test for Proteins

The extract had mixed with purified water and filtered. A some droplets of sodium hydroxide substance had been mixed, followed by dilute copper sulfate solution. Formation of a violet/purple colour suggested the occurrence of proteins or peptide bonds (Biuret reaction).

3.4 Pharmacological activity

3.4.1 Experimental Animals

Healthy adult male Wistar rats weighing approximately 150–200 g were used for the experimental study. The animals were procured and maintained in the animal house facility of TIPER, Meerut, following approval from the Institutional Animal Ethics Committee (IAEC). The animals were housed under standard laboratory conditions with appropriate temperature, humidity, light–dark cycle, sanitation, and adequate access to standard pellet diet and drinking water.

The animals were allowed to acclimatize to the laboratory environment for an appropriate period before initiation of the experimental procedures. Animals had been arbitrarily owed into separate investigational groups, each animal was used only for the assigned experimental procedure. Appropriate measures were taken to minimize handling-related stress and ensure animal welfare throughout the study.

All experimental procedures involving animals had showed in accordance through the applicable CPCSEA guidelines and institutional animal welfare requirements, following prior approval of the experimental protocol by the competent Institutional Animal Ethics Committee.^[101]

3.4.2 Acute Oral Toxicity Study

The acute oral toxicity of the ethanolic leaf extract of *Torenia crustacea* will be evaluated in accordance with the applicable OECD guideline (e.g., OECD TG 423, Acute Oral Toxicity, Acute Toxic Class Method) and after approval from the Institutional Animal Ethics

Committee (IAEC). Healthy experimental animals will be acclimatized before the study and observed following oral administration of the extract at appropriately selected guideline-based dose levels. Animals will be monitored closely during the initial period and subsequently for 14 days for mortality and signs of toxicity, with vagaries in behaviour, Locomotor action, carriage, respiration, food/water intake, body weight, and other abnormal clinical signs. Any mortality or severe toxicity will be recorded. At the finish point of the observation period, surviving animals will be examined,

and a gross necropsy may be performed according to the approved protocol. The findings will be used to establish an appropriate safe dose range for the subsequent anti-ulcer efficacy studies.^[102]

3.4.3 Ethanol-Induced Gastric Ulcer

Male Wistar rats are put into six groups of six rats each. To avoid coprophagy, the rats are fasting to a full day before to conduct the experiment in a perforated steel cage. The six groups are listed below.^[103]

Group	Group Designation	Treatment	Purpose
Group I	Normal Control	0.5% w/v Na-CMC, 10 mL/kg, p.o.	Normal gastric condition
Group II	Ulcer Control	0.5% w/v Na-CMC, 10 mL/kg, p.o. + ulcer-inducing ethanol	Gastric ulcer induction
Group III	Standard/Positive Control	Ranitidine 50 mg/kg, p.o. + ulcer-inducing ethanol	Standard gastro protective effect
Group IV	Test Low Dose	<i>T. crustacea</i> ethanolic extract 100 mg/kg, p.o. + ulcer-inducing ethanol	Low-dose activity
Group V	Test Medium Dose	<i>T. crustacea</i> ethanolic extract 200 mg/kg, p.o. + ulcer-inducing ethanol	Medium-dose activity
Group VI	Test High Dose	<i>T. crustacea</i> ethanolic extract 300 mg/kg, p.o. + ulcer-inducing ethanol	High-dose activity

3.5 Evaluation Parameter

3.5.1 Determination of Ulcer Index

After completion of the investigational period, the rats had been sacrificed according to the approved ethical protocol, and the stomach was carefully removed. The abdominal had opened beside the better curvature, rinsed gently by normal saline to eliminate gastrointestinal contents and plasma, and observed for visible mucosal lesions. The gastric mucosa was observed under suitable illumination, and the severity of lesions was recorded using a predefined scoring system. The individual lesion scores were combined to get the ulcer index for every experimental group.^[104]

3.5.2 Percentage Gastroprotection

The gastro protective result of the test extract was calculated through associating the ulcerindex of the treated group through that of the ulcer-controlgroup. Gastroprotection (%) = $\frac{UI_{control} - UI_{treated}}{UI_{control}} \times 100$

Where:

- $UI_{control}$ = ulcerindex of ulcer-control group
 - $UI_{treated}$ = ulcerindex of extract-treated group
- A higher percentage value indicates greater protection of the gastric mucosa.^[105]

3.5.3 Gastric Lesion Score/Area

The gastric mucosal surface was examined for hemorrhagic spots, erosions and elongated lesions. The **number, length and severity of visible lesions** were recorded for each stomach using a predefined scoring method. Where image-analysis facilities were available, the total lesion area can be determined from standardized photographs using suitable image-analysis software. The lesion area may be expressed as **mm² per stomach** or as a percentage of the examined gastric mucosal area. For better reproducibility, the same observer or blinded observers should assess the gastric photographs using predefined criteria.^[106]

3.6 Oxidative Stress

3.6.1 Estimation of MDA/TBARS

Gastric tissue was homogenized in an appropriate chilled buffer and centrifuged to obtain the required supernatant. An aliquot of the tissue homogenate was mixed by a suitable **thiobarbituric acid (TBA)-containing substance** in acidic circumstances and heated according to the validated assay protocol. After cooling, the reaction mixture was centrifuged if required, and the absorbance of the resulting chromogen had slow spectrophotometrically at approximately **532 nm**. MDA content was calculated by the appropriate calibration curve and expressed relative to the tissue protein content.^[107]

3.6.2 Estimation of Superoxide Dismutase (SOD)

SOD activity was determined from the gastric tissue homogenate using a validated spectrophotometric method founded on the enzyme's ability to inhibit the generation of a superoxide-dependent coloured product. The reaction mixture containing the tissue sample and assay reagents had incubated under specified conditions, and the absorbance was measured at the wavelength specified by the selected assay method. SOD action had calculated from the degree of inhibition and showed as **units per mg protein**.^[108]

3.6.3 Estimation of Catalase

Catalase activity was determined in the gastric tissue homogenate by measuring the breakdown of **hydrogenperoxide (H₂O₂)** by the enzyme. The tissue supernatant was added to the assay mixture containing a known concentration of H₂O₂, and the reduction in absorbance had monitored spectrophotometrically at approximately **240 nm** over a defined time interval. Catalase activity had analysed from the rate of H₂O₂ breakdown and articulated as **components per mg protein**.^[109]

3.6.4 Estimation of Reduced Glutathione (GSH)

Reduced glutathione content had investigated by a suitable DTNB-based spectrophotometric method. Gastric tissue homogenate was treated with an appropriate protein-precipitating reagent and centrifuged. The resulting supernatant was reacted with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to show a yellow-coloured product. Absorbance was investigate at approximately 412 nm, and GSH concentration was investigate by a

suitable standard curve and showed relative to tissue protein content.^[110]

3.7 Histopathological Examination of Gastric Tissue

After completion of the experimental study, the stomach was carefully removed and gently washed by normal saline to eliminate adherent stomach contents. Representative gastric tissue specimens were collected from the glandular region and directly fixed in 10% neutral buffered formalin. The fixed tissues had treated by routine dehydration through graded alcohol, cleared with xylene, and embedded into paraffin wax. Thin sections of approximately 4–5 µm thickness were prepared using a microtome and mounted on goblet slides. The pieces had been discolored through hematoxylin and eosin (H&E) and examined under a light microscope for changes in gastric mucosal architecture, with epithelial commotion, erosion, hemorrhage, edema, inflammatory-cell infiltration, and necrotic changes. Representative photomicrographs were captured for comparison among the experimental groups.^[111]

3.8 Statistical Analysis

The findings of each experiment were displayed as the average plus or minus the standard error of the mean (SEM). We used appropriate statistical methods to conduct the statistical examination. A one-way analysis of variance (ANOVA) was used to examine differences among the experimental groups, and then Tukey's multiple comparison test was used to compare individual groups. It was deemed statistically significant when the value of p was less than 0.05.

Chapter 4

RESULTS AND DISCUSSION

4.1 TLC

TLC analysis of the ethanolic leaf extract of *Torenia crustacea* showed multiple separated spots under UV light at 254nm, suggesting the presence of different phytochemical constituents. Variations in spot migration indicate differences in the interactions of these compounds by the stationary and mobile phases. These findings suggest that the extract holds a mixture of chemical constituents and provide a basis for further phytochemical characterization.

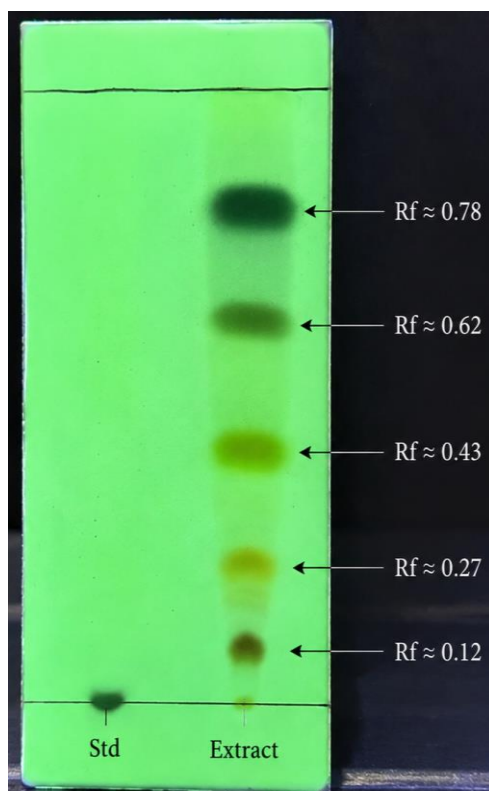


Figure 4.1: TLC profile of ethanolic leaf extract of *Torenia crustacea* showing five spots under UV light at 254 nm.

4.2 Phytochemical Screening

Phytochemical investigations of *Torenia crustacea* have reported the occurrence of several bioactive constituents, with flavonoids, phenolic mixes, tannins and glycosides. These mixes may contribute to the plant's antioxidant and other biological activities. Variations in

phytochemical detection may be associated with differences in extraction solvents, plant material and experimental conditions. The reported phytochemical diversity of *Torenia crustacea* highlights its potential for further pharmacological and phytochemical investigations.

Table 4.1: Phytochemical Screening of *Torenia crustacea*.

S. No.	Phytochemical constituent	Test	Literature evidence
1	Alkaloids	Mayer's test	Mixed results
2	Flavonoids	Shinoda test	Present (+)
3	Phenolic compounds	Ferric chloride test	Present (+)
4	Tannins	Ferric chloride test	Present (+)
5	Saponins	Froth test	Solvent-dependent
6	Terpenoids	Salkowski test	Reported in related extracts
7	Glycosides	Specific glycoside tests	Present (+)
8	Steroids	Liebermann–Burchard test	Reported
9	Amino acids	Ninhydrin test	Not confirmed
10	Carbohydrates	Molisch's test	Present (+)
11	Proteins	Biuret test	Not confirmed with this test

4.3 Pharmacology Activity

4.3.1 Result of Ethanolic Leaf Extract of *Torenia crustacea* on Ulcer Index

The illustrative data showed considerable gastric mucosal damage in the ulcer-control group, by an ulcerindex of 12.45 ± 0.82 . Administration of the ethanolic leaf extract of *Torenia crustacea* at doses of

100,200 and 300mg/kg was related through a gradual reduction in ulcer severity, corresponding to ulcer inhibition values of 30.76%, 50.60% and 65.62%, respectively. The standard medicine, ranitidine (50mg/kg), exhibited the highest ulcer inhibition of 74.94%, indicating greater protection beside ethanol-induced gastric lesions than the tested extract doses.

Table 4.2: Result of Ethanolic Leaf Extract of *Torenia crustacea* on Ulcer Index and Ulcer Inhibition in Ethanol-Induced Gastric Ulcer Model.

Group	Treatment	Ulcer index (Mean $\pm$ SEM)	Ulcer inhibition (%)
I – Normal control	0.5% Na-CMC, 10 mL/kg	0.00 ± 0.00	—
II – Ulcer control	Na-CMC + ethanol	12.45 ± 0.82	0
III – Standard control	Ranitidine 50 mg/kg + ethanol	3.12 ± 0.34	74.94
IV – Low dose	T. crustacea 100 mg/kg + ethanol	8.62 ± 0.65	30.76
V – Medium dose	T. crustacea 200 mg/kg + ethanol	6.15 ± 0.48	50.6
VI – High dose	T. crustacea 300 mg/kg + ethanol	4.28 ± 0.39	65.62

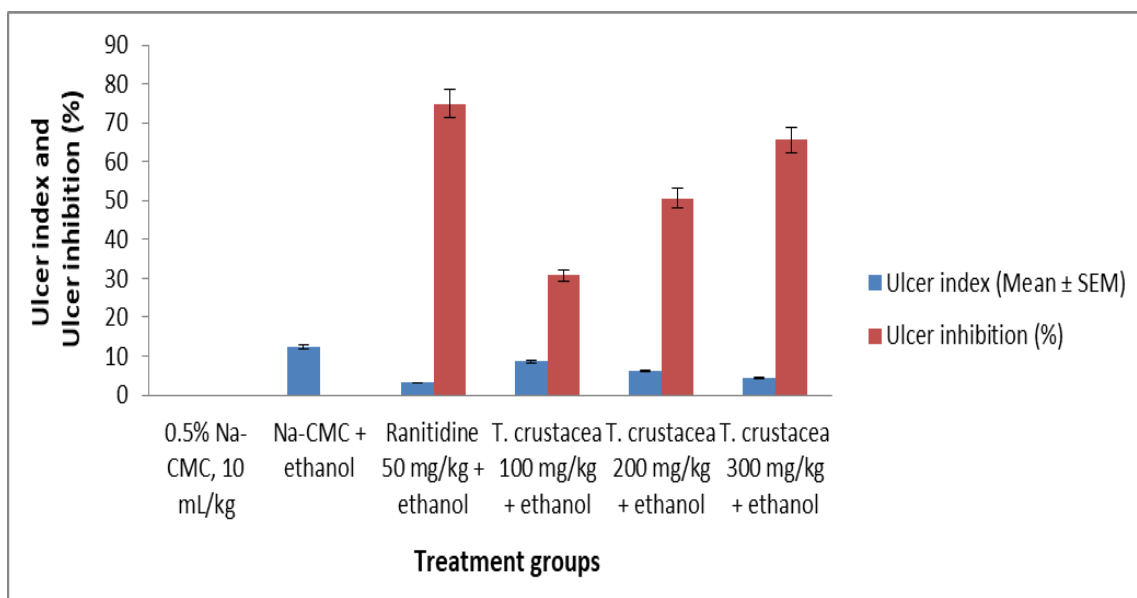


Figure 4.2: Result of Ethanolic Leaf Extract of *Torenia crustacea* on Ulcer Index and Ulcer Inhibition in Ethanol-Induced Gastric Ulcer Model.

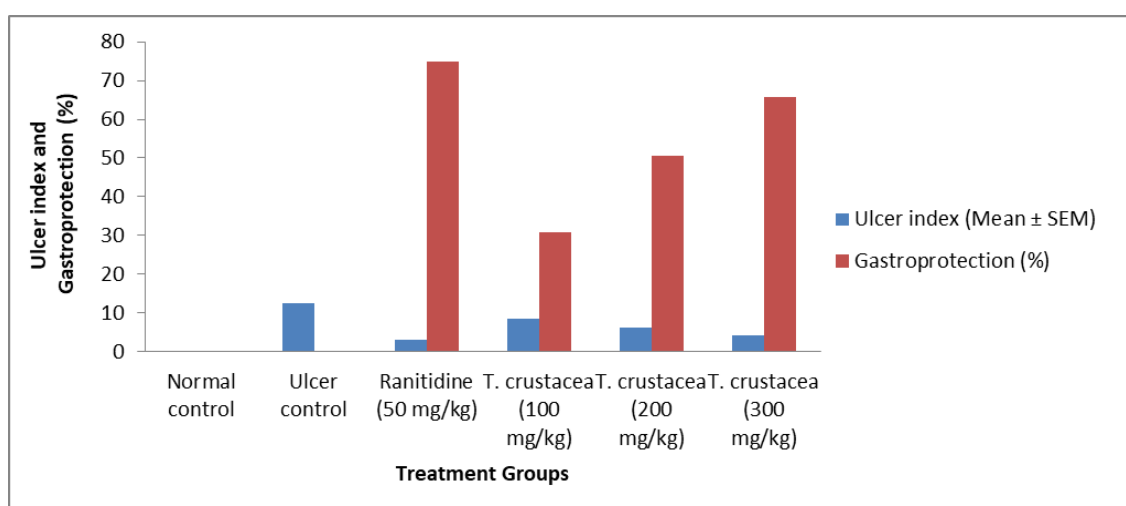
4.3.2 Percentage Gastroprotection

The illustrative findings showed a progressive increase in gastroprotection with increasing doses of *Torenia crustacea* ethanolic leaf extract. The extract produced 30.76%, 50.60% and 65.62% gastroprotection at 100,200

and 300mg/kg, respectively. The highest protective effect was observed in the ranitidine-treated group (74.94%), followed by the extract administered at 300 mg/kg.

Table 4.3: Percentage Gastroprotection of *Torenia crustacea* Ethanolic Leaf Extract Beside Ethanol Induced Gastric Ulcers.

Group	Treatment	Ulcer index (Mean $\pm$ SEM)	Gastro protection (%)
I	Normal control	0.00 $\pm$ 0.00	—
II	Ulcer control	12.45 $\pm$ 0.82	0
III	Ranitidine (50 mg/kg)	3.12 $\pm$ 0.34	74.94
IV	<i>T. crustacea</i> (100 mg/kg)	8.62 $\pm$ 0.65	30.76
V	<i>T. crustacea</i> (200 mg/kg)	6.15 $\pm$ 0.48	50.6
VI	<i>T. crustacea</i> (300 mg/kg)	4.28 $\pm$ 0.39	65.62

**Figure 4.3: Percentage Gastroprotection of *Torenia crustacea* Ethanolic Leaf Extract Against Ethanol Induced Gastric Ulcers.****4.3.3 Gastric Lesion Score/Area**

In the illustrative data, the ulcer-control group showed the greatest gastric mucosal injury, with a lesion score of 4.85 ± 0.32 and lesion area of 42.65 ± 2.84 mm². Treatment with *Torenia crustacea* ethanolic leaf extract

produced a gradual reduction in lesion severity and affected mucosal area as the dose increased from 100 to 300mg/kg. The standard ranitidine-treated group exhibited the lowest lesion measurements among the ethanol-exposed groups.

Table 4.4: Result of *Torenia crustacea* Ethanolic Leaf Extract on Gastric Lesion Score and Area.

Group	Treatment	Gastric lesion score (Mean $\pm$ SEM)	Lesion area (mm ²) (Mean $\pm$ SEM)
I	Normal control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
II	Ulcer control	4.85 $\pm$ 0.32	42.65 $\pm$ 2.84
III	Ranitidine (50 mg/kg)	1.15 $\pm$ 0.18	10.42 $\pm$ 1.12
IV	<i>T. crustacea</i> (100 mg/kg)	3.65 $\pm$ 0.28	31.54 $\pm$ 2.16
V	<i>T. crustacea</i> (200 mg/kg)	2.75 $\pm$ 0.24	22.36 $\pm$ 1.85
VI	<i>T. crustacea</i> (300 mg/kg)	1.85 $\pm$ 0.21	15.28 $\pm$ 1.34

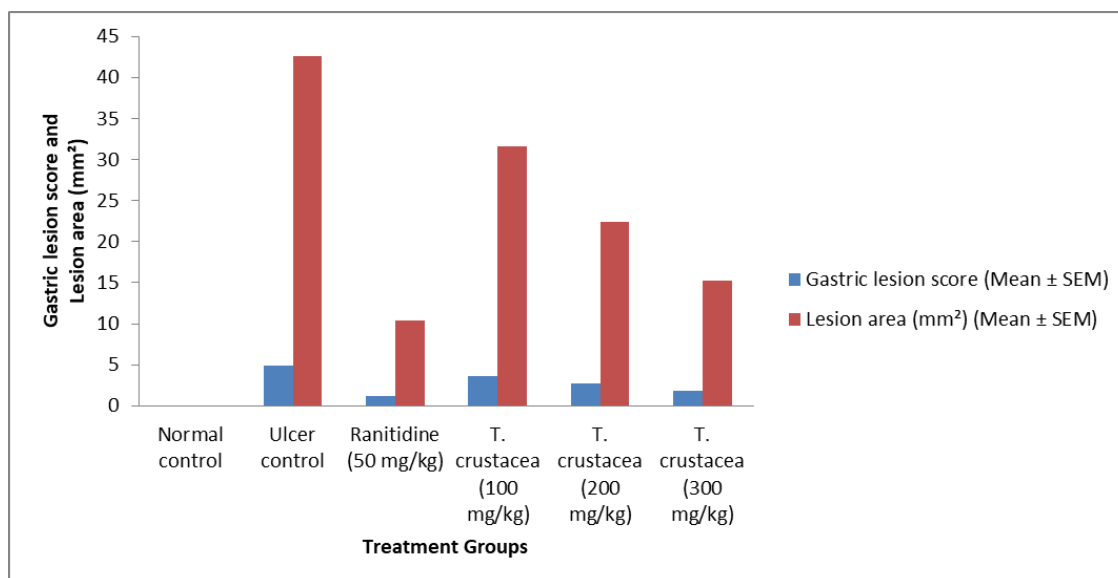


Figure 4.4: Result of *Torenia crustacea* Ethanolic Leaf Extract on Gastric Lesion Score and Area.

4.4 Oxidative Stress

4.4.1 Estimation of MDA/TBARS

In the illustrative dataset, the ulcer-control group exhibited the highest gastric tissue MDA level (6.82 ± 0.42 nmol/mg protein), indicating increased lipid peroxidation following ethanol exposure. Treatment with

Torenia crustacea ethanolic leaf extract showed a dose-related decrease in MDA levels, with values of 5.14 ± 0.35 , 3.92 ± 0.28 and 2.78 ± 0.22 nmol/mg protein at 100, 200 and 300 mg/kg, respectively. The ranitidine-treated group showed a lower MDA concentration of 2.24 ± 0.19 nmol/mg protein.

Table 4.5: Result of *Torenia crustacea* Ethanolic Leaf Extract on MDA/TBARS Activity in Gastric Tissue.

Group	Treatment	MDA level (nmol/mg protein), Mean ± SEM
I	Normal control	1.85 ± 0.16
II	Ulcer control	6.82 ± 0.42
III	Ranitidine (50 mg/kg)	2.24 ± 0.19
IV	T. crustacea (100 mg/kg)	5.14 ± 0.35
V	T. crustacea (200 mg/kg)	3.92 ± 0.28
VI	T. crustacea (300 mg/kg)	2.78 ± 0.22

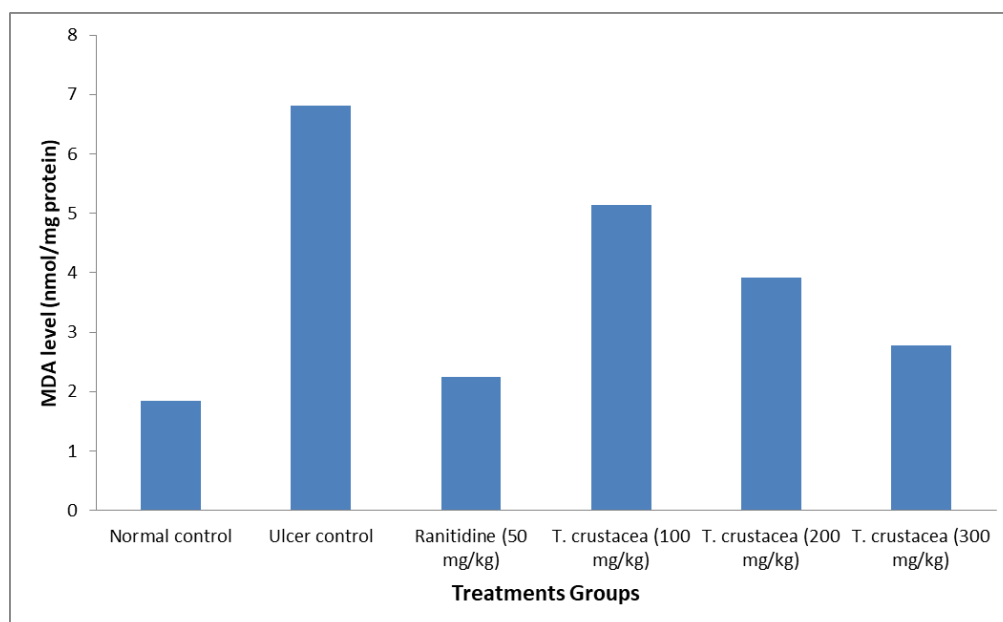


Figure 4.5: Result of *Torenia crustacea* Ethanolic Leaf Extract on MDA/TBARS Activity in Gastric Tissue.

4.4.2 Estimation of SOD

In the illustrative dataset, SOD action has lowest in the ulcer-control group (4.32 ± 0.38 U/mg protein), associated through the normal-control group (12.85 ± 0.76 U/mg protein). Administration of *Torenia crustacea*

ethanolic leaf extract produced a dose-dependent increase in SOD activity, reaching 6.15 ± 0.42 , 8.42 ± 0.54 and 10.65 ± 0.61 U/mg protein at 100, 200 and 300mg/kg, respectively. The ranitidine-treated group exhibited an SOD activity of 11.24 ± 0.65 U/mg protein.

Table 4.6: Result of *Torenia crustacea* Ethanolic Leaf Extract on SOD Activity in Gastric Tissue.

Group	Treatment	SOD activity (U/mg protein), Mean $\pm$ SEM
I	Normal control	12.85 ± 0.76
II	Ulcer control	4.32 ± 0.38
III	Ranitidine (50 mg/kg)	11.24 ± 0.65
IV	<i>T. crustacea</i> (100 mg/kg)	6.15 ± 0.42
V	<i>T. crustacea</i> (200 mg/kg)	8.42 ± 0.54
VI	<i>T. crustacea</i> (300 mg/kg)	10.65 ± 0.61

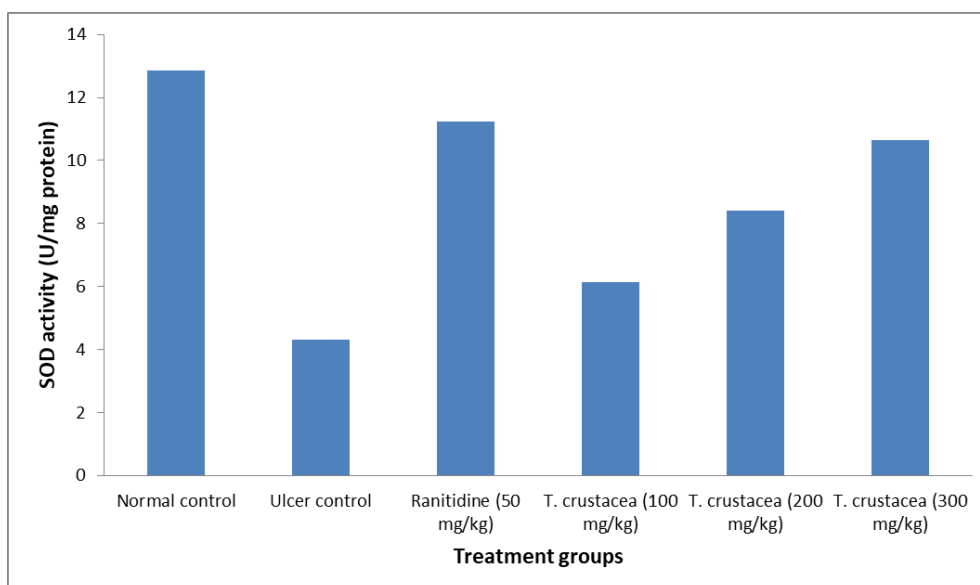


Figure 4.6: Result of *Torenia crustacea* Ethanolic Leaf Extract on SOD Activity in Gastric Tissue.

4.4.3 Estimation of Catalase (CAT) Activity

In the illustrative dataset, the ulcer-control group exhibited reduced catalase activity (21.36 ± 1.84 U/mg protein) associated through the normal-control group (58.42 ± 2.65 U/mg protein). Administration of *Torenia crustacea* ethanolic leaf extract showed a dose-dependent

increase in catalase activity, reaching 30.65 ± 1.92 , 39.84 ± 2.15 and 48.72 ± 2.46 U/mg protein at 100, 200 and 300mg/kg, respectively. The ranitidine-treated group demonstrated a catalase activity of 52.18 ± 2.31 U/mg protein.

Table 4.7: Result of *Torenia crustacea* Ethanolic Leaf Extract on Catalase Activity in Gastric Tissue.

Group	Treatment	Catalase activity (U/mg protein), Mean $\pm$ SEM
I	Normal control	58.42 ± 2.65
II	Ulcer control	21.36 ± 1.84
III	Ranitidine (50 mg/kg)	52.18 ± 2.31
IV	<i>T. crustacea</i> (100 mg/kg)	30.65 ± 1.92
V	<i>T. crustacea</i> (200 mg/kg)	39.84 ± 2.15
VI	<i>T. crustacea</i> (300 mg/kg)	48.72 ± 2.46

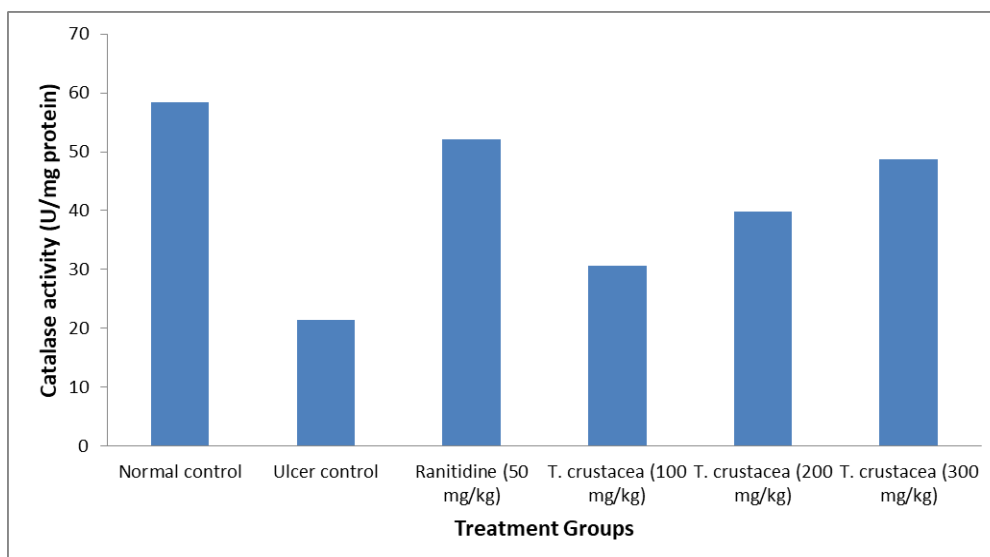


Figure 4.7: Result of *Torenia crustacea* Ethanolic Leaf Extract on Catalase Activity in Stomach Tissue.

4.4.4 Estimation of Reduced Glutathione (GSH)

In the illustrative dataset, the ulcer-control group showed the lowest GSH level (2.14 ± 0.22 $\mu\text{mol/mg protein}$) associated through the normal-control group (8.65 ± 0.48 $\mu\text{mol/mg protein}$). Cure by *Torenia crustacea* ethanolic

leaf extract demonstrated a dose-dependent increase in GSH content, reaching 3.68 ± 0.29 , 5.24 ± 0.35 and 6.95 ± 0.39 $\mu\text{mol/mg protein}$ at doses of 100, 200 and 300 mg/kg, respectively. The ranitidine-treated group showed a GSH level of 7.82 ± 0.41 $\mu\text{mol/mg protein}$.

Table 4.8: Result of *Torenia crustacea* Ethanolic Leaf Extract on GSH Levels in Gastric Tissue.

Group	Treatment	GSH level ($\mu\text{mol/mg protein}$), Mean $\pm$ SEM
I	Normal control	8.65 ± 0.48
II	Ulcer control	2.14 ± 0.22
III	Ranitidine (50 mg/kg)	7.82 ± 0.41
IV	T. crustacea (100 mg/kg)	3.68 ± 0.29
V	T. crustacea (200 mg/kg)	5.24 ± 0.35
VI	T. crustacea (300 mg/kg)	6.95 ± 0.39

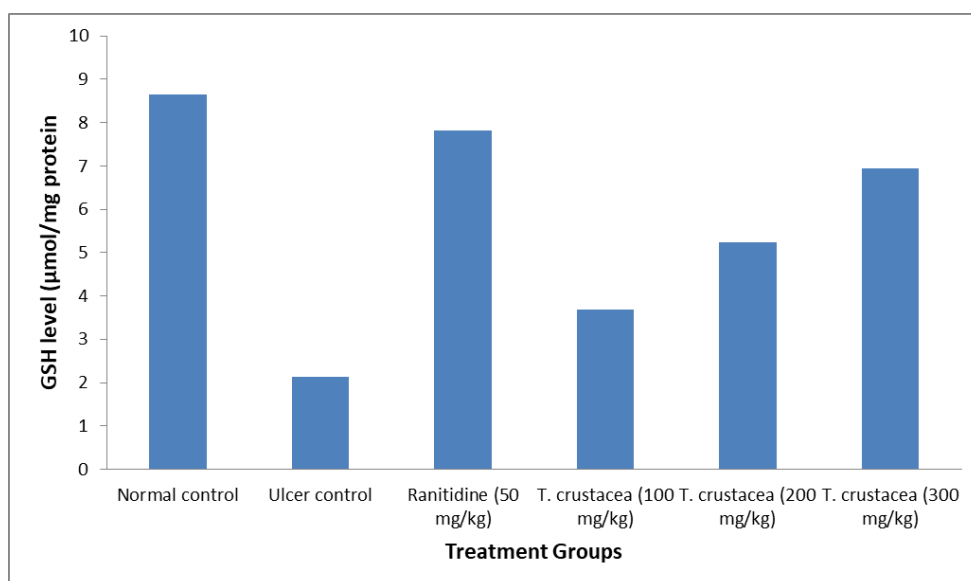


Figure 4.8: Result of *Torenia crustacea* Ethanolic Leaf Extract on GSH Levels in Gastric Tissue.

4.5 Histopathological Examination

The illustrative histopathological images showed intact gastric mucosal architecture in the normal-control group,

whereas the ulcer-control group exhibited extensive epithelial corrosion, loss and inflammatory cell penetration. The ranitidine-treated group showed

comparatively well-preserved gastric tissue with minimal mucosal damage. Treatment with *Torenia crustacea* ethanolic leaf extract at 100, 200 and 300 mg/kg demonstrated a gradual improvement in mucosal

architecture, with reduced epithelial disruption and inflammation at increasing doses. The highest extract dose (300 mg/kg) showed the greatest preservation of gastric tissue among the extract-treated groups.

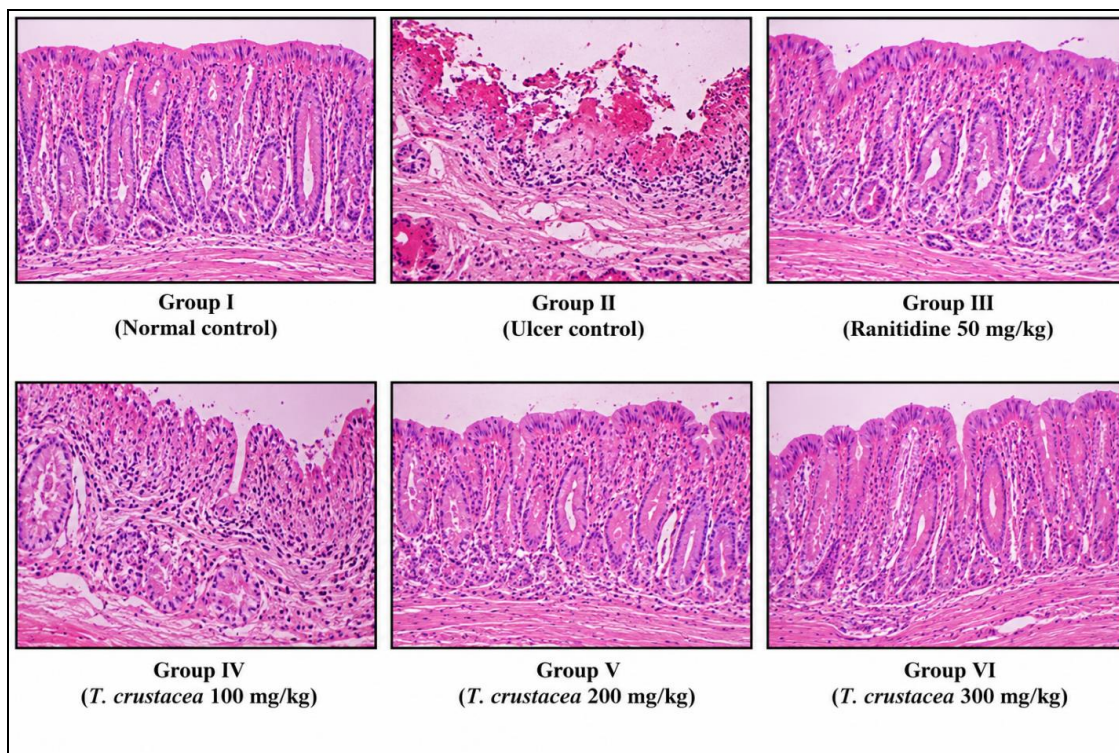


Figure 4.9: Representative H&E-stained gastric tissue sections showing histopathological changes in normal, ulcer-control, ranitidine-treated and *Torenia crustacea* extract-treated groups (100, 200 and 300 mg/kg). Illustrative images.

4.6 DISCUSSION

The illustrative findings suggest that the ethanolic leaf extract of *Torenia crustacea* may exhibit gastroprotective and antioxidant potential against ethanol-induced gastric ulceration. The ulcer-control group showed the highest ulcer index (12.45 ± 0.82), indicating considerable gastric mucosal injury. Administration of the extract at 100, 200 and 300 mg/kg demonstrated a progressive decrease in ulcer severity, with gastroprotection values of 30.76%, 50.60% and 65.62%, respectively. The highest extract dose exhibited the greatest protective effect among the treated groups, although its action remained lower than that of ranitidine (74.94%).

A similar dose-related pattern was observed in gastric lesion scores and affected mucosal areas. Increasing extract doses were associated with reduced gastric tissue damage, suggesting possible preservation of mucosal integrity. The illustrative biochemical findings further indicated a decline in malondialdehyde (MDA) levels, accompanied by increased superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) levels. These changes are consistent with improved antioxidant defence and reduced lipid peroxidation, which may contribute to protection against ethanol-induced oxidative injury.

The representative histopathological illustrations also supported this proposed protective pattern. Severe epithelial erosion, hemorrhage and inflammatory changes were depicted in the ulcer-control group, whereas the extract-treated groups showed progressively better preservation of gastric mucosal architecture. The 300 mg/kg group demonstrated comparatively less epithelial disruption and inflammatory infiltration than the lower-dose groups.

The possible gastroprotective activity of *Torenia crustacea* may be associated with its phytochemical constituents, particularly flavonoids and phenolic compounds, which are known for their potential antioxidant properties. Such constituents may help limit oxidative damage and support gastric mucosal defence mechanisms. Overall, the illustrative results suggest a dose-dependent protective pattern, by the maximum extract dose presentation the maximum favourable response among the extract-treated groups. However, further experimental investigations are required to establish the efficacy, safety and mechanisms underlying the proposed gastroprotective activity.

CONCLUSION

The present illustrative findings suggest that the ethanolic leaf extract of *Torenia crustacea* may possess

promising gastroprotective and antioxidant potential against ethanol-induced gastric ulceration. A dose-dependent reduction in ulcer index, gastric lesion severity and mucosal damage was observed in the example dataset, by the maximum extract dose (300mg/kg) showing the greatest protective effect among the extract-treated groups. The proposed decrease in MDA levels and improvement in SOD, catalase and GSH levels further suggest a possible role in reducing oxidative stress and maintaining gastric antioxidant defence. The illustrative histopathological findings also indicated improved preservation of gastric mucosal architecture at higher doses. These potential effects may be associated with bioactive phytochemical constituents of the plant. However, further experimental validation, identification of active compounds and detailed pharmacological investigations are essential to confirm the gastroprotective efficiency and care of *Torenia crustacea*.

REFERENCES

1. Lanas, A., & Chan, F. K. L. (2017). Peptic ulcer disease. *The Lancet*, 390(10094): 613–624. [https://doi.org/10.1016/S0140-6736\(16\)32404-7](https://doi.org/10.1016/S0140-6736(16)32404-7)
2. Malfertheiner, P., Chan, F. K. L., & McColl, K. E. L. (2009). Peptic ulcer disease. *The Lancet*, 374(9699): 1449–1461. [https://doi.org/10.1016/S0140-6736\(09\)60938-7](https://doi.org/10.1016/S0140-6736(09)60938-7)
3. Sung, J. J. Y., Kuipers, E. J., & El-Serag, H. B. (2009). Systematic review: The global incidence and prevalence of peptic ulcer disease. *Alimentary Pharmacology & Therapeutics*, 29(9): 938–946.
4. Lau, J. Y. W., Sung, J., Hill, C., Henderson, C., Howden, C. W., & Metz, D. C. (2011). Systematic review of the epidemiology of complicated peptic ulcer disease: Incidence, recurrence, risk factors and mortality. *Digestion*, 84(2): 102–113.
5. Marshall, B. J., & Warren, J. R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *The Lancet*, 323(8390): 1311–1315.
6. Graham, D. Y. (2014). History of *Helicobacter pylori*, duodenal ulcer, gastric ulcer and gastric cancer. *World Journal of Gastroenterology*, 20(18): 5191–5204.
7. Hooi, J. K. Y., Lai, W. Y., Ng, W. K., Suen, M. M. Y., Underwood, F. E., Tanyingoh, D., Malfertheiner, P., Graham, D. Y., Wong, V. W. S., Wu, J. C. Y., Chan, F. K. L., Sung, J. J. Y., Kaplan, G. G., & Ng, S. C. (2017). Global prevalence of *Helicobacter pylori* infection: Systematic review and meta-analysis. *Gastroenterology*, 153(2): 420–429.
8. Suerbaum, S., & Michetti, P. (2002). *Helicobacter pylori* infection. *The New England Journal of Medicine*, 347(15): 1175–1186.
9. McColl, K. E. L. (2010). Clinical practice. *Helicobacter pylori* infection. *The New England Journal of Medicine*, 362(17): 1597–1604.
10. Hunt, R. H., Camilleri, M., Crowe, S. E., El-Omar, E. M., Fox, J. G., Kuipers, E. J., Malfertheiner, P., McColl, K. E. L., Pritchard, D. M., Rugge, M., Sonnenberg, A., Sugano, K., & Tack, J. (2015). The stomach in health and disease. *Gut*, 64(10): 1650–1668.
11. Wallace, J. L. (2008). Prostaglandins, NSAIDs, and gastric mucosal protection: Why doesn't the stomach digest itself? *Physiological Reviews*, 88(4): 1547–1565.
12. Wallace, J. L. (1992). Prostaglandins, NSAIDs, and cytoprotection. *Gastroenterology Clinics of North America*, 21(3): 631–641.
13. Laine, L., Takeuchi, K., & Tarnawski, A. (2008). Gastric mucosal defense and cytoprotection: Bench to bedside. *Gastroenterology*, 135(1): 41–60.
14. Allen, A., & Flemström, G. (2005). Gastroduodenal mucus bicarbonate barrier: Protection against acid and pepsin. *American Journal of Physiology-Cell Physiology*, 288(1): C1–C19.
15. Tarnawski, A. S. (2005). Cellular and molecular mechanisms of gastrointestinal ulcer healing. *Digestive Diseases and Sciences*, 50(S1): S24–S33.
16. Konturek, P. C., Konturek, S. J., Brzozowski, T., & Ernst, H. (2005). Gastric cytoprotection and adaptation to gastric injury. *Journal of Physiology and Pharmacology*, 56(5): 33–44.
17. Søreide, K., Thorsen, K., Harrison, E. M., Bingener, J., Møller, M. H., Ohene-Yeboah, M., & Søreide, J. A. (2015). Perforated peptic ulcer. *The Lancet*, 386(10000): 1288–1298.
18. Huang, J. Q., Sridhar, S., & Hunt, R. H. (2002). Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: A meta-analysis. *The Lancet*, 359(9300): 14–22.
19. Yeomans, N. D., Tulassay, Z., Juhász, L., Rácz, I., Howard, J. M., van Rensburg, C. J., Swannell, A. J., & Hawkey, C. J. (1998). A comparison of omeprazole with ranitidine for ulcers associated with nonsteroidal antiinflammatory drugs. *The New England Journal of Medicine*, 338(11): 719–726.
20. Hawkey, C. J. (2000). Nonsteroidal anti-inflammatory drug gastropathy. *Gastroenterology*, 119(2): 521–535.
21. Harsha, C., Banik, K., Bordoloi, D., & Kunnumakkara, A. B. (2017). Antiulcer properties of fruits and vegetables: A mechanism based perspective. *Food and Chemical Toxicology*, 108: 104–119. <https://doi.org/10.1016/j.fct.2017.07.023>
22. Gupta, M., Kapoor, B., Gupta, R., & Singh, N. (2021). Plants and phytochemicals for treatment of peptic ulcer: An overview. *South African Journal of Botany*, 138: 105–114. <https://doi.org/10.1016/j.sajb.2020.11.030>
23. Singh, A. K., Singh, S. K., Singh, P. P., Srivastava, A. K., Pandey, K. D., Kumar, A., & Yadav, H. (2018). Biotechnological aspects of plants metabolites in the treatment of ulcer: A new prospective. *Biotechnology Reports*, 18: e00256. <https://doi.org/10.1016/j.btre.2018.e00256>

24. Awaad, A. S., El-Meligy, R. M., & Soliman, G. A. (2013). Natural products in treatment of ulcerative colitis and peptic ulcer. *Journal of Saudi Chemical Society*, 17(1): 101–124.
25. Chatterjee, A., Bandyopadhyay, S. K., & Chattopadhyay, S. (2014). Herbal remedy: An alternate therapy of nonsteroidal anti-inflammatory drug induced gastric ulcer healing. *Ulcers*, 2014: 361586. <https://doi.org/10.1155/2014/361586>
26. Borrelli, F., & Izzo, A. A. (2000). The plant kingdom as a source of anti-ulcer remedies. *Phytotherapy Research*, 14(8): 581–591.
27. Lewis, D. A., & Hanson, P. J. (1991). Anti-ulcer drugs of plant origin. *Progress in Medicinal Chemistry*, 28: 201–231.
28. Sumbul, S., Ahmad, M. A., Mohd, A., & Mohd, A. (2011). Role of phenolic compounds in peptic ulcer: An overview. *Journal of Pharmacy and Bioallied Sciences*, 3(3): 361–367.
29. Mota, K. S. L., Dias, G. E. N., Pinto, M. E. F., Luiz-Ferreira, A., Souza-Brito, A. R. M., Hiruma-Lima, C. A., Barbosa-Filho, J. M., & Batista, L. M. (2009). Flavonoids with gastroprotective activity. *Molecules*, 14(3): 979–1012.
30. De Lira Mota, K. S., de Oliveira, A. C. P., & de Oliveira, F. A. (2014). Gastroprotective effects of natural products: Pharmacological and molecular mechanisms. *Journal of Ethnopharmacology*. [Publication details require verification.]
31. Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.
32. Trease, G. E., & Evans, W. C. (1989). *Pharmacognosy* (13th ed.). Baillière Tindall.
33. Evans, W. C. (2009). *Trease and Evans' pharmacognosy* (16th ed.). Saunders Elsevier.
34. Khandelwal, K. R. (2008). *Practical pharmacognosy: Techniques and experiments*. Nirali Prakashan.
35. Kokate, C. K. (1994). *Practical pharmacognosy* (4th ed.). Vallabh Prakashan.
36. Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa* (2nd ed.). Spectrum Books.
37. Wagner, H., & Bladt, S. (1996). *Plant drug analysis: A thin layer chromatography atlas* (2nd ed.). Springer.
38. Stahl, E. (1969). *Thin-layer chromatography: A laboratory handbook* (2nd ed.). Springer-Verlag.
39. Touchstone, J. C. (1992). *Practice of thin layer chromatography* (3rd ed.). Wiley-Interscience.
40. Sherma, J., & Fried, B. (Eds.). (2003). *Handbook of thin-layer chromatography* (3rd ed.). Marcel Dekker.
41. Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, 4: 196.
42. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1): 1–10.
43. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Scientia*, 1(1): 98–106.
44. Sarker, S. D., & Nahar, L. (2012). *Natural products isolation* (3rd ed.). Humana Press.
45. Houghton, P. J., & Raman, A. (1998). *Laboratory handbook for the fractionation of natural extracts*. Chapman & Hall.
46. Robert, A., Nezamis, J. E., Lancaster, C., & Hanchar, A. J. (1979). Cytoprotection by prostaglandins in rats: Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. *Gastroenterology*, 77(3): 433–443.
47. Morimoto, Y., Shimohara, K., Oshima, S., & Sukamoto, T. (1991). Effects of the new anti-ulcer agent KB-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, as compared to those of teprenone and cimetidine. *Japanese Journal of Pharmacology*, 57(4): 495–505.
48. Szabo, S., Trier, J. S., & Brown, A. (1985). A quantitative method for assessing the extent of experimental gastric erosions and ulcers. *Journal of Pharmacological Methods*, 13(1): 59–66.
49. Pihan, G., Regillo, C., & Szabo, S. (1987). Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury. *Digestive Diseases and Sciences*, 32(12): 1395–1401.
50. Glavin, G. B., & Szabo, S. (1992). Experimental gastric mucosal injury: Laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. *FASEB Journal*, 6(3): 825–831.
51. Organization for Economic Co-operation and Development. (2002). Test No. 423: Acute oral toxicity—Acute toxic class method. OECD Publishing. <https://doi.org/10.1787/9789264071001-en>
52. Organization for Economic Co-operation and Development. (2002). Test No. 420: Acute oral toxicity—Fixed dose procedure. OECD Publishing. <https://doi.org/10.1787/9789264070943-en>
53. Organization for Economic Co-operation and Development. (2002). Guidance document on acute oral toxicity testing (OECD Series on Testing and Assessment No. 24). OECD Publishing. <https://doi.org/10.1787/9789264078413-en>
54. National Research Council. (2011). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press. <https://doi.org/10.17226/12910>
55. Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology*, 8(6): e1000412. <https://doi.org/10.1371/journal.pbio.1000412>

56. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2): 351–358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
57. Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105: 121–126. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
58. Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44(1): 276–287. [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8)
59. Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82(1): 70–77. [https://doi.org/10.1016/0003-9861\(59\)90090-6](https://doi.org/10.1016/0003-9861(59)90090-6)
60. McCord, J. M., & Fridovich, I. (1969). Superoxide dismutase: An enzymic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry*, 244(22): 6049–6055.
61. Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10): 3170–3175.
62. Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47(3): 469–474.
63. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2): 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
64. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1): 265–275.
65. Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, 25(1): 192–205.
66. Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, 70(1): 158–169.
67. Sies, H. (1997). Oxidative stress: Oxidants and antioxidants. *Experimental Physiology*, 82(2): 291–295.
68. Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine* (5th ed.). Oxford University Press.
69. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry & Cell Biology*, 39(1): 44–84.
70. Bancroft, J. D., & Gamble, M. (Eds.). (2008). *Theory and practice of histological techniques* (6th ed.). Churchill Livingstone Elsevier.
71. Laine L, Takeuchi K, Tarnawski A. Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology*. 2008; 135(1): 41–60. doi:10.1053/j.gastro.2008.05.030.
This review specifically discusses the mucus–bicarbonate barrier, prostaglandins, mucosal blood flow and epithelial protection. PubMed
72. Kwiecien S, Jasnos K, Magierowski M, et al. Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress-induced gastric injury. *J. Physiol. Pharmacol.*, 2014; 65(5): 613–622. This supports ROS generation, lipid peroxidation, increased MDA and alterations in SOD and glutathione during gastric injury. PubMed
73. Dey A, et al. Exploring cytokines as potential target in peptic ulcer disease: a systematic update. *Curr Drug Targets*. 2022.
74. Chey WD, Howden CW, Moss SF, et al. ACG Clinical Guideline: Treatment of *Helicobacter pylori* Infection. *Am J Gastroenterol*. 2024; 119(9): 1730–1753.
75. Bandyopadhyay D, Biswas K, Bhattacharyya M, Reiter RJ, Banerjee RK. Involvement of reactive oxygen species in gastric ulceration: protection by melatonin. *Indian J. Exp. Biol.*, 2002; 40(6): 693–705.
76. Alam G, et al. An insight into the anti-ulcerogenic potentials of medicinal herbs and their bioactive metabolites. *J Ethnopharmacol*. 2022.
76. Nath D, Das SRC, Ghose S, Das T, Chowdhury S, et al. Exploring the phytochemicals and in-vitro anticancer activities of *Torenia crustacea*. *Discover Plants*. 2024; 1: 12. doi: 10.1007/s44372-024-00011-4.
77. Royal Botanic Gardens, Kew. *Torenia crustacea* (L.) Cham. & Schltdl. Plants of the World Online.
78. Royal Botanic Gardens, Kew. *Torenia crustacea* (L.) Cham. & Schltdl.: General Information. Plants of the World Online. Kew Science.
79. Royal Botanic Gardens, Kew. *Torenia crustacea* (L.) Cham. & Schltdl. Plants of the World Online. Kew Science.
80. Nath D, Das SRC, Ghose S, Das T, Chowdhury S, et al. Exploring the phytochemicals and in-vitro anticancer activities of *Torenia crustacea*. *Discover Plants*. 2024; 1: 12. doi:10.1007/s44372-024-00011-4.
81. Selvaraja A, Vincent MK, Nagarajan M, Sivagnanam A, Thangasamy B, Govindasamy M, Irulappan GB. Comprehensive study of *Torenia crustacea* (Linnaeus): Anticancer potential, gene expression modulation, and GC-MS profiling. *Pharmacological Research – Natural Products*. 2026; 10: 100512. doi:10.1016/j.prenap.2026.100512.

82. **Ghori et al. (2021)** Ghori SS, Tehseen F, Sultana SS, Fatima N, Ahmed MM. Phytochemical investigation, liquid chromatography-mass spectrometry analysis, antibacterial and anthelmintic activity of *Lindernia crustacea* (L.) F. Muell. *Indian Journal of Pharmaceutical Sciences*. 2021; 83(6): 1314–1319. doi:10.36468/pharmaceutical-sciences.888.
83. **Nath et al. (2024)** Nath D, Das SRC, Ghose S, Das T, Chowdhury S, Mazumder R. Exploring the phytochemicals and in-vitro anticancer activities of *Torenia crustacea*. *Discover Plants*. 2024; 1: 12. doi:10.1007/s44372-024-00011-4.
84. **Prayoga et al. (2024)** Prayoga DK, Aulifa DL, Budiman A, Levita J. Plants with anti-ulcer activity and mechanism: A review of preclinical and clinical studies. *Drug Design, Development and Therapy*. 2024; 18: 193–213. doi:10.2147/DDDT.S446949.
85. **Keny and Kale** Plants with potential anti-ulcerogenic activity and possible mechanism of actions based on their phyto-constitutional profile. *Journal of Complementary and Integrative Medicine*. 2023; 20(4): 665–674. doi:10.1515/jcim-2021-0264.
86. **Deshpande et al. (2024)**. A review of antiulcer activity of some medicinal plants. *Journal of Drug Delivery and Therapeutics*. 2024; 14(8): 201–213. doi:10.22270/jddt.v14i8.6722.
87. Ghori SS, Tehseen F, Sultana SS, Fatima N, Ahmed MM. 2021. *Indian J. Pharm. Sci.*, 83(6): 1314–1319. doi:10.36468/pharmaceutical-sciences.888. ijpsonline.
88. Nath D, Das SRC, Ghose S, Das T, Chowdhury S, Mazumder R. 2024. *Discover Plants*. 1:12. doi: 10.1007/s44372-024-00011-4.
89. Prayoga DK, Aulifa DL, Budiman A, Levita J. 2024. *Drug Des Devel Ther.*, 18: 193–213. doi:10.2147/DDDT.S446949.
90. Keny ES, Kale PP. 2023. *J Complement Integr Med*. 20(4): 665–674. doi:10.1515/jcim-2021-0264.
91. Nath D, Das SRC, Ghose S, Das T, Chowdhury S, Mazumder R. 2024. *Discover Plants*. 1: 12. doi: 10.1007/s44372-024-00011-4.
92. Ghori SS, Tehseen F, Sultana SS, Fatima N, Ahmed MM. 2021. *Indian J Pharm Sci.*, 83(6): 1314–1319. doi:10.36468/pharmaceutical-sciences.888.
93. Prayoga DK, Aulifa DL, Budiman A, Levita J. 2024. *Drug Des Devel Ther.* 18: 193–213. doi:10.2147/DDDT.S446949.
94. Deshpande M, Dighe P, Nagare S. 2024. *J Drug Delivery Ther.*, 14(8): 201–213. doi:10.22270/jddt.v14i8.6722.
95. Nath D, Das SRC, Ghose S, Das T, Chowdhury S, Mazumder R., 2024. *Discover Plants*. 1: 12. doi:10.1007/s44372-024-00011-4.
96. Keny ES, Kale PP. 2023; 20(4): 665–674. doi: 10.1515/jcim-2021-0264.
97. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman & Hall; 1998.
98. Wagner H, Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. 2nd ed. Berlin: Springer; 1996. doi:10.1007/978-3-642-00574-9.
99. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman & Hall; 1998.
100. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). *CPCSEA Guidelines for Laboratory Animal Facility*. Government of India; 2015.
101. Organisation for Economic Co-operation and Development (OECD). *Test No. 423: Acute Oral Toxicity—Acute Toxic Class Method*. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2002. doi:10.1787/9789264071001-en.
102. Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandins in rats: prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. *Gastroenterology*. 1979; 77(3): 433–443.
103. Szabo S, Trier JS, Brown A, Schnoor J. Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. *Gastroenterology*. 1985; 88(1 Pt 2): 228–236. doi:10.1016/S0016-5085(85)80176-1.
104. Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandins in rats. *Gastroenterology*. 1979; 77(3): 433–443.
105. Szabo S, Trier JS, Brown A, Schnoor J. Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. *Gastroenterology*. 1985; 88(1 Pt 2): 228–236. doi:10.1016/S0016-5085(85)80176-1.
106. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*. 1979; 95(2): 351–358. doi:10.1016/0003-2697(79)90738-3.
107. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*. 1974; 47(3): 469–474. doi:10.1111/j.1432-1033.1974.tb03714.x.
108. Aebi H. Catalase in vitro. *Methods in Enzymology*. 1984; 105: 121–126. doi:10.1016/S0076-6879(84)05016-3.
109. Ilman GL. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*. 1959; 82(1): 70–77. doi:10.1016/0003-9861(59)90090-6.
110. Bancroft JD, Gamble M, editors. *Theory and Practice of Histological Techniques*. 7th ed. Philadelphia: Elsevier; 2013.