ANTIOXIDANT EFFECTS OF *Sida acuta* ON INDOMETHACIN-INDUCED ULCEROGENIC RATS

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CERTIFICATION

This is to certify that the project work was successfully carried out by **OKUNLOLA HANNAH TEMITOPE HND/23/SLT/FT/0211, ADESHINA ARIKE ABISOLA HND/23/SLT/FT/0348, RAMON TOHEEB ADETUNJI HND/23/SLT/FT/0449, AJIBOYE JOSHUA OLUWADAMILARE HND/23/SLT/FT/0544, OKUNOLA ESTHER MOFOLASHADEMI HND/23/SLT/FT/0714 and OYEBODE KAOSARAT OPEYEMI HND/23/SLT/FT/0818** and it was read and approved as meeting the requirements of Department of Science Laboratory Technology and has been prepared in accordance with the regulation governing the preparation and presentation of Kwara State Polytechnic, Ilorin.

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DEDICATION

This work is dedicated to Almighty God, the Alpha and Omega of all wisdom and understanding that made it possible and given us the opportunity to complete our Higher National Diploma (HND) without any bad records

And also to our lovely parents, sisters and brothers whose unwavering support and encouragement mean the world to us.

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ABSTRACT

Gastric ulceration is a common gastrointestinal disorder often associated with oxidative stress, inflammation, and mucosal damage. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are known to induce gastric lesions by inhibiting prostaglandin synthesis and promoting the generation of reactive oxygen species (ROS). This study investigates the protective effect of Sida acuta, a medicinal plant known for its antioxidant properties, on oxidative stress markers and lipid peroxidation in indomethacin-induced ulcerogenic rats.

Fifty two (52) adult Wistar rats were divided into seven groups: a normal control, an ulcer control (indomethacin only), two treatment groups receiving different doses (200 and 400 mg/kg) of Sida acuta extract, and a standard group treated with omeprazole (20 mg/kg). Gastric ulcers were induced by a single dose of indomethacin (30 mg/kg). After 7 days of treatment, gastric tissues were harvested and analyzed for enzymatic antioxidants—superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH)—as well as malondialdehyde (MDA), a marker of lipid peroxidation.

The results showed that indomethacin significantly reduced antioxidant enzyme levels and increased MDA concentration compared to the control group. Treatment with Sida acuta extract significantly restored antioxidant levels and reduced MDA concentration in a dose-dependent manner, comparable to the standard omeprazole group.

These findings suggest that Sida acuta possesses potent antioxidant and gastroprotective properties, likely due to its ability to scavenge free radicals and inhibit lipid peroxidation. The plant extract may serve as a natural therapeutic agent for managing NSAID-induced gastric ulcers.

CHAPTER ONE

1.0 INTRODUCTION

Modern scientific investigations have confirmed that *Sida acuta* possesses a variety of pharmacological properties, largely attributed to its rich content of bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and saponins. Given its documented antioxidant, anti-inflammatory, and antimicrobial activities, there is increasing scientific interest in exploring *Sida acuta* for the treatment of gastrointestinal disorders, particularly those involving oxidative stress and mucosal injury. This study aims to evaluate the protective effects of *Sida acuta* on antioxidant enzyme activity and lipid peroxidation in Indomethacin-induced gastric ulcer models. Understanding these effects may provide insights into the therapeutic potential of the plant as a natural alternative or adjunct in ulcer management *(Femoe et al., 2022).*

1.1 Background of the Study

Peptic ulcer disease (PUD) is a chronic, relapsing condition that affects millions of individuals globally and remains a significant public health concern. It is characterized by mucosal erosions in the stomach or duodenum, resulting from an imbalance between aggressive factors such as gastric acid, *Helicobacter pylori* (*H. pylori*) infection, and nonsteroidal anti-inflammatory drugs (NSAIDs)and the protective mechanisms of the gastrointestinal mucosa. Among the major contributors to PUD, *H. pylori*, a Gram-negative, helical-shaped, microaerophilic bacterium, plays a pivotal role in the pathogenesis of various gastrointestinal conditions (Mladenova, 2021). The bacterium has evolved specialized mechanisms to survive in the acidic environment of the stomach, including the production of

urease, which neutralizes gastric acid, and the ability to adhere to the gastric epithelium, leading to inflammation and mucosal damage *(Hunt et al., 2011).*

Globally, nearly 50% of the population is estimated to be infected with *H. pylori*, although the prevalence varies with geographic location, socio-economic status, hygiene practices, and age (*Malaoa, 2021).* The infection is most commonly acquired in childhood and persists unless effectively treated. The World Health Organization (WHO) has classified *H. pylori* as a Group I carcinogen due to its established link with gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (Mladenova, 2021). The bacterium is transmitted via multiple pathways, including fecal-oral, oral-oral, and gastro-oral routes, and is particularly prevalent in populations with inadequate sanitation, overcrowded living conditions, and limited access to clean water *(Seth et al., 2013).*

Standard treatment regimens for *H. pylori*-associated ulcers typically involve the use of triple or quadruple therapy, which includes a proton pump inhibitor (PPI) and two or more antibiotics. However, these conventional therapies are increasingly facing challenges, such as poor patient compliance, side effects (e.g., nausea, diarrhea, and dysbiosis), antibiotic resistance, and ulcer recurrence *(Dharmani & Palit, 2006).* The incomplete success of conventional treatments has driven a growing interest in alternative and complementary therapies, especially those derived from medicinal plants. One such promising plant is *Sida acuta*, a perennial shrub belonging to the family Malvaceae. It is widely distributed in tropical and subtropical regions and is known locally as “broom weed” or “stubborn weed.” Traditionally, it has been used in ethnomedicine for the treatment of various ailments, including fever, malaria, wounds, and inflammation *(Usman & Abdulkarim, 2023).*

Gastric and duodenal ulcers are common gastrointestinal disorders that can result in serious complications such as bleeding, perforation, and gastric outlet obstruction. These ulcers arise primarily due to an imbalance between aggressive factors like gastric acid secretion, *H. pylori* infection, NSAID use, bile acids, and pepsin, and protective mechanisms such as mucus and bicarbonate secretion, mucosal blood flow, and the production of endogenous prostaglandins. The increasing prevalence of gastric ulcers globally has been attributed to lifestyle changes, stress, irregular eating habits, increased NSAID consumption, and antibiotic-resistant *H. pylori* strains *(Zatorski, 2017).*

Indomethacin is an indole derivative, non-steroidal, anti-inflammatory drug with anti-inflammatory, analgesic, and antipyretic effects. It is used in the treatment of ankylosing spondylitis, osteoarthritis, rheumatoid arthritis, gout arthritis, bursitis, tendonitis, synovitis, and other inflammatory diseases because of its effective suppression of pain, fever, color, and *edema (Botting, 2006; Suleyman et al., 2010).*

It is known that the inhibition potencies of non-steroidal anti-inflammatory drugs (NSAIDs) on cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes are different. It is believed that while inhibition of COX1 by NSAIDs causes side effects as a result of reduced prostaglandin (PG) synthesis, inhibition of COX-2 is related to their anti-inflammatory effect (Suleyman et al., 2008). Indomethacin potently damages PG synthesis by inhibiting both the COX-1 and COX-2 enzymes. Inhibition of the COX-1 and COX-2 enzymes is necessary for gastric damage to occur (Suleyman et al., 2007). Indomethacin and similar NSAIDs, which inhibit both isoforms of the COX enzyme, produce more severe damage in gastric tissue, even gastrointestinal bleeding when combined with antithrombotic

agents. Inhibition of the COX-2 enzyme is thought to be responsible for indomethacin's anti-inflammatory effect, while inhibition of COX-1 is responsible for its gastrointestinal system (GIS) side effects *(Delaney et al., 2007).*

Indomethacin became the first-choice drug to produce an experimental ulcer model as a result of having a higher ulcerogenic potential than other NSAIDs. The fact that nimesulide, which is less selective for COX-2, is able to inhibit NSAID-induced gastric damage, while celecoxib and rofecoxib, which are more selective for COX-2 (350 to 800 times as selective), are unable to inhibit these ulcers, reveals that it is impossible to attribute the GIS side effects of indomethacin and other NSAIDs to the inhibition of only the COX-1 enzyme *(Adewoye and Salami, 2013).*

In light of these issues, there is a growing interest in natural compounds with antioxidant and anti-inflammatory properties that can restore the balance between oxidative damage and antioxidant defense. Medicinal plants offer a rich source of such compounds and have been used for centuries in traditional medicine to treat ulcers and other inflammatory conditions. Among these, *Sida acuta* has garnered attention due to its wide range of pharmacological effects and its traditional use in treating gastrointestinal complaints *(Asusheyi et al., 2010)*

Phytochemical analyses of *Sida acuta* have confirmed the presence of bioactive constituents such as flavonoids, known for their strong antioxidant potential. These compounds neutralize free radicals, inhibit lipid peroxidation, and enhance the activity of endogenous antioxidant enzymes (Femoe et al., 2022). Furthermore, flavonoids and other polyphenolic compounds in *Sida acuta* may possess antiulcerogenic effects by reinforcing the mucosal barrier, inhibiting inflammatory cytokines, and modulating gastric acid secretion. Several in vivo studies have demonstrated the efficacy of plant extracts in protecting against NSAID-induced

gastric ulcers in animal models. These studies report reductions in ulcer index, restoration of antioxidant enzyme levels, and histological improvements in the gastric mucosa. The affordability, accessibility, and minimal side effects associated with medicinal plants make them attractive options for populations with limited access to modern healthcare systems *(Yousef et al., 2019).*

Therefore, the current study seeks to investigate the protective effects of *Sida acuta* against indomethacin-induced gastric ulcers, with particular focus on its influence on antioxidant defense mechanisms and lipid peroxidation. The findings from this research may not only validate the traditional use of *Sida acuta* in ulcer treatment but also contribute to the development of novel, plant-based therapies with fewer adverse effects and greater accessibility.

1.2 Statement of the Problem

Peptic ulcer disease, particularly those caused by *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs (NSAIDs) like Indomethacin, continues to be a major global health challenge. While conventional treatments such as proton pump inhibitors and antibiotics have proven effective, their long-term use is often accompanied by adverse effects, drug resistance, and recurrence of ulcers. Furthermore, NSAID-induced gastric ulcers, including those caused by Indomethacin, result from both prostaglandin inhibition and oxidative stress due to free radical production, which significantly impairs gastric mucosal integrity.

Despite numerous pharmacological advancements, a safe, effective, and affordable treatment for Indomethacin-induced ulcers that addresses both mucosal injury and oxidative stress remains elusive. Medicinal plants, including *Sida acuta*, offer promising alternatives due to their antioxidant and cytoprotective properties. However, there is limited scientific data on the specific effects of *Sida acuta* on

antioxidant enzyme modulation and lipid peroxidation in Indomethacin-induced ulcer models. This lack of data hampers the integration of such herbal therapies into mainstream ulcer management.

1.3 Justification of the Study

Gastric ulcers caused by NSAIDs such as Indomethacin remain a pressing medical concern due to their frequency and the limitations associated with current treatment modalities. These limitations include drug toxicity, recurrence, and increasing resistance to conventional antibiotics used in *H. pylori*-associated ulcer therapy. Furthermore, the role of oxidative stress in ulcerogenesis is well-documented, yet few therapies directly address the oxidative imbalance associated with mucosal injury. The exploration of medicinal plants like *Sida acuta*, which has a rich history in traditional medicine, provides a promising avenue for identifying natural, cost-effective, and safer alternatives to synthetic drugs. Preliminary studies have shown that *Sida acuta* possesses significant antioxidant and anti-inflammatory properties, but its role in treating Indomethacin-induced gastric ulcers has not been extensively explored or validated through scientific research.

This study is justified by the need to develop more accessible and well-tolerated treatments for peptic ulcers, particularly in resource-limited settings. If proven effective, *Sida acuta* could offer a natural therapeutic option that reduces oxidative stress, enhances antioxidant defense, and protects the gastric mucosa, thereby contributing significantly to the management of NSAID-induced ulcers and promoting the use of scientifically supported herbal medicine.

1.4 Aim and objectives of the Study

The aim of this study is to evaluate the protective effects of *Sida acuta* extract on antioxidant status and lipid peroxidation in indomethacin-induced ulcer models.

Objectives of the Study

- To determine the extent of gastric mucosal damage caused by Indomethacin administration in experimental models.
- To assess the antioxidant enzyme activity (such as superoxide dismutase, catalase, and glutathione peroxidase) in gastric tissues following treatment with *Sida acuta* extract.
- To evaluate the level of lipid peroxidation (via malondialdehyde levels) in gastric tissues of treated and untreated ulcer models.
- To compare the anti-ulcerogenic efficacy of *Sida acuta* with a standard anti-ulcer drug (omeprazole).
- To analyze the phytochemical constituents of *Sida acuta* responsible for its gastroprotective and antioxidant effects.

CHAPTER TWO

2.0 LITERATURE REVIEW

Over time, a wide range of treatment approaches has emerged, encompassing both traditional remedies and contemporary pharmacological solutions. The introduction of proton pump inhibitors (PPIs) marked a significant breakthrough in the management of ulcers by efficiently suppressing gastric acid secretion and facilitating mucosal healing *(Sung et al., 2020).* Although H2-receptor antagonists like ranitidine were once widely used, they have been largely superseded by PPIs due to their greater therapeutic effectiveness (Laine et al., 2021). The identification of *Helicobacter pylori* as a major contributor to peptic ulcer disease was a pivotal moment, leading to the development of antibiotic-based treatments that markedly enhanced clinical outcomes (Malfertheiner et al., 2017). Nonetheless, the increasing prevalence of antibiotic resistance presents a significant obstacle, driving the need for continued exploration of alternative therapeutic options *(Sung et al., 2020).*

Contemporary research has increasingly highlighted the gut microbiome's involvement in ulcer pathogenesis, indicating that variations in microbial populations can impact both disease progression and the healing process (Lanas and Chan, 2017). In addition, regenerative therapies—such as those utilizing stem cells—are being investigated for their potential in treating chronic ulcers, with encouraging findings related to tissue regeneration and recovery (Liesegang, 2019). The field of phytomedicine has also made strides, with ongoing studies examining natural bioactive compounds like flavonoids and polyphenols for their anti-ulcerogenic properties, which may serve as supportive treatment options (*Malfertheiner et al., 2017).* Despite these medical advances, ulcers continue to be a pressing public health issue globally, particularly in low-resource settings where

access to healthcare and *H. pylori* eradication programs remains limited. To bridge these gaps, further research is essential in order to create more effective and widely accessible therapies *(Sung et al., 2020).*

Ulcers have posed a major medical challenge for centuries, prompting extensive research into their causes, underlying mechanisms, and treatment strategies. Among these, peptic ulcers are the most thoroughly investigated, primarily affecting the gastrointestinal tract due to *Helicobacter pylori* infection and the long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) *(Malfertheiner et al., 2017).* Studies suggest that ulcer development results from a disruption in the balance between harmful factors—such as gastric acid secretion and pepsin activity—and the protective defenses of the mucosal lining *(Laine et al., 2021).* Additionally, the influence of stress and dietary patterns in ulcer development has been examined, though their direct role in causation remains a subject of ongoing debate *(Lanas and Chan, 2017).*

2.1 Ulcer

Ulcers are characterized as open sores or lesions that form on the skin or mucosal surfaces, triggered by a range of etiological factors, including infections, chronic medical conditions, and drug-induced tissue damage (Lanas and Chan, 2017). These lesions can manifest in various areas of the body, such as the gastrointestinal tract—specifically as gastric and duodenal ulcers—the skin (as pressure ulcers), and the oral cavity (aphthous ulcers). Among these types, peptic ulcers, especially those affecting the stomach and duodenum, have received considerable attention due to their considerable burden on global health systems *(Sung et al., 2020).* The global prevalence of ulcers is influenced by factors such as dietary practices, the availability of healthcare services, and the rate of *Helicobacter*

pylori infections. In developing nations, the incidence of peptic ulcers remains elevated, largely attributed to inadequate sanitation and high *H. pylori* transmission rates *(Sung et al., 2020).* Conversely, industrialized countries have experienced a decrease in ulcer cases, a trend credited to enhanced medical care and the extensive use of proton pump inhibitors (PPIs) *(Lanas and Chan, 2017)*. Nonetheless, the widespread use of nonsteroidal anti-inflammatory drugs (NSAIDs), particularly among older adults, continues to be a major contributor to ulcer development *(Laine et al., 2021).*

Over time, treatment strategies for ulcers have evolved significantly, ranging from traditional herbal remedies to advanced pharmacological therapies. The development of PPIs marked a turning point in ulcer management by effectively inhibiting gastric acid secretion and supporting mucosal repair (*Sung et al., 2020*). Previously, H2-receptor antagonists like ranitidine were commonly used, but their popularity has waned due to the greater efficacy of PPIs (Laine et al., 2021). The identification of *H. pylori* as a primary causative agent in peptic ulcer disease led to the implementation of antibiotic regimens, dramatically enhancing therapeutic success rates (Malfertheiner et al., 2017). However, the emergence of antibiotic-resistant strains has introduced new challenges, underscoring the need for continued research into alternative treatment approaches *(Sung et al., 2020).*

Recent research has increasingly emphasized the gut microbiome's role in the pathophysiology of ulcers, indicating that the composition of gut microbes can significantly affect both disease progression and the healing process (Lanas and Chan, 2017). In parallel, regenerative medicine approaches—particularly those involving stem cell therapy—have been explored for managing chronic ulcers, demonstrating encouraging outcomes in terms of tissue regeneration and recovery (Liesegang, 2019). The field of phytomedicine has also advanced, leading to studies

on natural compounds with anti-ulcerogenic effects, such as flavonoids and polyphenols, which may serve as valuable adjunct therapies *(Malfertheiner et al., 2017).*

The socioeconomic impact of ulcers continues to be considerable, driven by the high costs of managing chronic cases and addressing complications like gastrointestinal bleeding and perforation (Sung et al., 2020). Psychological factors have also been implicated, with mounting evidence linking chronic stress to increased gastric mucosal vulnerability and ulcer development (*Laine et al., 2021).* Furthermore, lifestyle habits—particularly alcohol consumption and smoking—have been identified as major risk factors, known to impair ulcer healing and raise the likelihood of recurrence *(Lanas and Chan, 2017).*

Despite progress in understanding and treating ulcers, they remain a significant public health issue worldwide, especially in low-resource settings where access to healthcare services and *H. pylori* eradication initiatives is limited. Addressing these disparities requires further investigation and the development of more accessible and effective treatment modalities (Sung et al., 2020). Looking ahead, future studies should aim to innovate in therapeutic development, exploring options such as probiotics, stem cell-based therapies, and gene editing technologies to improve healing outcomes and reduce relapse rates *(Malfertheiner et al., 2017).*

2.2 Types of Ulcer

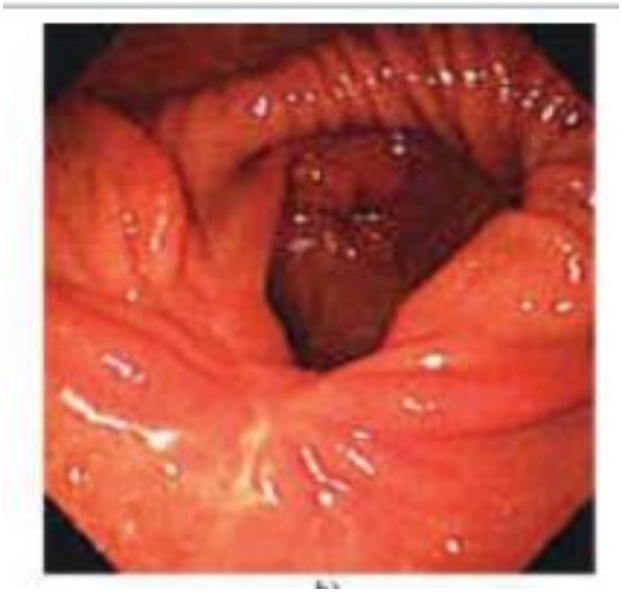
Ulcers are sores or open wounds that can occur on the skin or mucous membranes within the body. There are several types of ulcers, categorized based on their location and cause. Below are the main types:

2.2.1 Peptic Ulcer

Peptic ulcer disease is a gastrointestinal condition marked by mucosal injury caused by the corrosive effects of gastric acid and pepsin. It most commonly affects the stomach and proximal duodenum but can also present, though less frequently, in the lower esophagus, distal duodenum, or jejunum. These atypical cases are often associated with hypersecretory conditions such as Zollinger-Ellison syndrome, the presence of Cameron ulcers in hiatal hernias, or ectopic gastric mucosa found in anomalies like Meckel's diverticulum (Kalyanakrishnan et al., 2007). According to a systematic review of data from the United States, United Kingdom, and Europe, the annual incidence of peptic ulcer disease is approximately 1 to 2 per 1,000 individuals (Sung et al., 2009). This incidence appears to be on the decline, likely due to the reduced prevalence of *Helicobacter pylori* infection *(Agréus et al., 2016).*

Historically, *H. pylori* infection has been linked to approximately 90% of duodenal ulcers and 70% of gastric ulcers, although more recent findings suggest these figures may now be lower. Nonetheless, *H. pylori* remains a critical risk factor, not only for ulcer formation but also for the development of gastric cancer, highlighting the ongoing importance of eradication efforts (Ford et al., 2017). The two leading causes of peptic ulcer disease are *H. pylori* infection and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Kurata and Nogawa, 1997). While *H. pylori* is present in the gastroduodenal lining of most individuals with duodenal ulcers, only about 10 to 15 percent of those infected go on to develop ulcers. The bacterium adheres to the gastric mucosa, and its pathogenicity is heightened by the presence of an outer inflammatory protein and a functional cytotoxin-associated gene (cag) island within its genome, both of which contribute to its ulcerogenic potential (Nilsson et al., 2003). Infected individuals typically exhibit elevated basal and meal-induced gastrin secretion, along with diminished production of gastric

mucus and duodenal bicarbonate—conditions that collectively promote ulcer development *(Nilsson et al., 2003).*



Source: lauret (2015)

2.2.2 Skin Ulcer

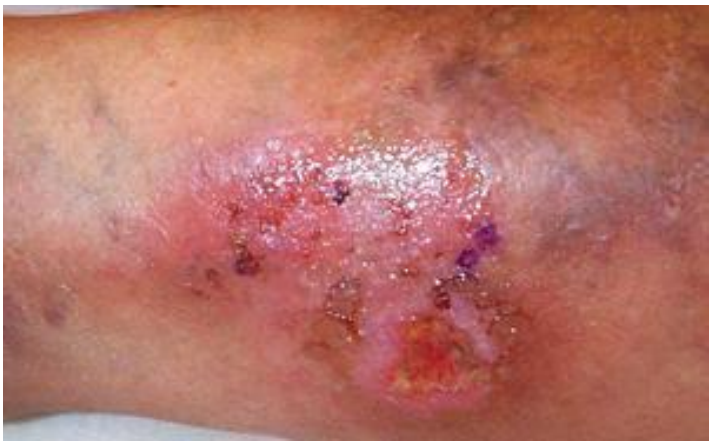
A skin ulcer is a localized defect or excavation of the skin surface that results from the progressive disintegration and necrosis of epidermal and dermal tissues, often extending into the subcutaneous layers *(Gupta et al., 2020).* These ulcers are caused by various underlying conditions, including poor circulation, prolonged pressure, infections, and metabolic disorders. Skin ulcers are classified based on their etiology, such as pressure ulcers, venous ulcers, arterial ulcers, neuropathic ulcers, and infectious ulcers *(Bergstrom et al., 2018).* Skin ulcers are a significant health concern globally, affecting individuals across various age groups and socioeconomic backgrounds. These chronic wounds result from multiple underlying conditions, including vascular insufficiency, prolonged pressure, infection, and

systemic diseases *(Sen et al., 2009).* Skin ulcers are characterized by the loss of epidermal and dermal integrity, leading to exposure of underlying tissues, which can predispose affected individuals to infections and prolonged healing processes *(Guo and DiPietro, 2010).*

The burden of skin ulcers is extensive, with epidemiological studies indicating their high prevalence, particularly among elderly individuals and those with chronic illnesses such as diabetes and peripheral vascular diseases *(Nussbaum et al., 2018).* The chronic nature of skin ulcers poses significant challenges in healthcare management, requiring long-term care and multidisciplinary approaches to mitigate their impact on patients' quality of life *(Mustoe et al., 2006).* The process of ulcer formation is often linked to impaired wound healing mechanisms. Normal wound healing involves hemostasis, inflammation, proliferation, and remodeling, but these processes are disrupted in chronic ulcers (Martin, 1997). Studies have shown that factors such as persistent inflammation, excessive protease activity, and reduced cellular migration contribute to the delayed healing seen in ulcerative conditions *(Eming et al., 2014).* Additionally, microbial colonization and biofilm formation further complicate wound healing, necessitating advanced therapeutic strategies to enhance tissue repair *(Bjarnsholt et al., 2008).*

Skin ulcers are also associated with substantial economic burdens, as their treatment often involves prolonged hospitalization, use of advanced wound care products, and surgical interventions *(Sen et al., 2009)*. A study by Guest *et al. (2015)* highlighted the financial implications of managing chronic wounds, emphasizing the need for cost-effective and innovative wound care solutions. An ulcer is a sore on the skin or a mucous membrane, accompanied by the disintegration of tissue. Ulcers can result in complete loss of the epidermis and often portions of the dermis and even subcutaneous fat. Ulcers are most common on the skin of the lower extremities

and in the gastrointestinal tract *(Kumar et al., 2004).* An ulcer that appears on the skin is often visible as an inflamed tissue with area of reddened skin. A skin ulcer is often visible in the event of exposure to heat or cold, irritation, or a problem with blood circulation. They can also be caused due to a lack of mobility, which causes prolonged pressure on the tissues. This stress in the blood circulation is transformed to a skin ulcer, commonly known as bedsores or decubitus ulcers. Ulcers often become infected, and pus forms *(Kumar et al., 2004).* Skin ulcers appear as open craters, often round, with layers of skin that have eroded. The skin around the ulcer may be red, swollen, and tender. Patients may feel pain on the skin around the ulcer, and fluid may ooze from the ulcer. In so cases, ulcers can bleed and, rarely, patients experience fever. Ulcers sometimes seem not to heal; healing, if it does occur, tends to be slow. Ulcers that heal within 12 weeks are usually classified as acute, and longer-lasting ones chronic *(Bella et al., 2024).*



Source: kuma (2004)

2.2.3 Mouth Ulcer

The oral cavity is lined with epithelial tissue that stretches from the inner surfaces of the lips to the oropharynx. This lining consists of both keratinized and non-keratinized mucosa. Keratinized areas include the dorsal surface of the tongue, gingivae, and hard palate, whereas non-keratinized mucosa is found on the labial and buccal mucosa, the ventral tongue, the floor of the mouth, and the soft palate. Alterations in the oral mucosa may serve as indicators of systemic diseases and can sometimes represent the earliest signs of an undiagnosed underlying condition *(Yogarajah and Setterfield, 2021).*

Oral ulcers are a frequent clinical finding. Although trauma or recurrent aphthous stomatitis are the most common causes, ulcers can also signal systemic illnesses or malignancies, such as oral cancer *(Scully and Felix, 2005).* Oral ulceration represents a common symptom across a broad spectrum of diseases, with diverse etiologies. Their diagnosis can be complex due to overlapping clinical and histopathological features. Most ulcerative oral lesions fall into one of four categories: infectious, immune-mediated, traumatic, or neoplastic *(Fitzpatrick et al., 2019).*

Traumatic ulcers are most often caused by physical injury to the mucosa. However, they can also result from local irritants, including topical Indomethacin, cocaine use, or crack cocaine smoking, particularly affecting the palate (Porter and Leao). Rarely, intranasal cocaine use can lead to ischemic necrosis involving the floor of the nose, progressing to hard palate ulceration and oronasal fistula formation. Additionally, local radiotherapy and certain cytotoxic chemotherapy agents may cause oral mucositis, which presents as painful mucosal erythema, ulceration, and epithelial sloughing *(Scully et al., 2004).*

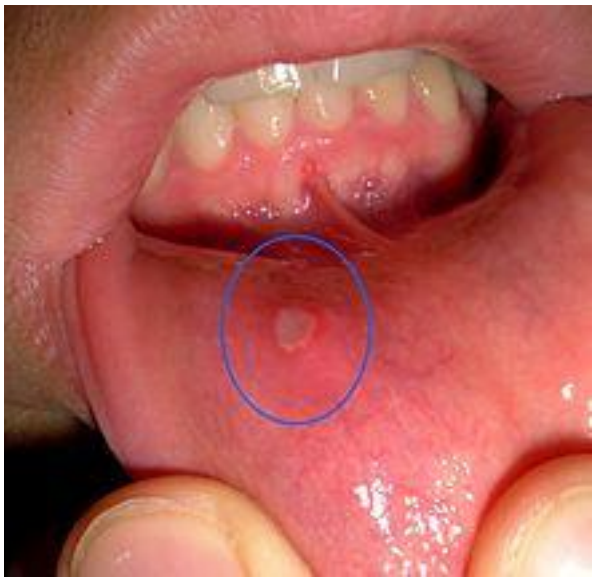
The exact mechanism behind mucositis is not fully understood, though it is thought to stem from impaired basal cell proliferation rather than alterations in the oral microbiota, such as increases in Gram-negative bacteria like *Enterobacteriaceae* (Stokman et al., 2003). Managing oral mucositis remains challenging. While benzydamine hydrochloride mouth rinses or sprays may offer symptom relief, opioid analgesics are often necessary for adequate pain control. Although chlorhexidine gluconate is widely used in clinical settings, it does not significantly improve mucositis symptoms. Emerging therapies under early clinical evaluation include granulocyte-macrophage colony-stimulating factor (GM-CSF) and protegrins *(Chen et al., 2000; Mantovani et al., 2003).*

Aphthous ulcers are the most common type of oral ulcer and generally resolve within 10 to 14 days without treatment. These lesions typically appear as small, round or oval ulcers with a pseudomembrane center and an erythematous halo, primarily affecting non-keratinized mucosa. Current treatments focus on symptom relief rather than promoting tissue regeneration. Oral rinses, while soothing due to their hydrating properties, do not accelerate healing. Even those containing antibiotics, antihistamines, antifungals, steroids, or anesthetics offer no significant advantage over saline mouthwashes in promoting recovery *(Dhanshri et al., 2024).*

Herpetiform ulcers, a subtype of aphthous ulcers, are so named due to their resemblance to herpes lesions, although they are not caused by the herpes virus and are non-contagious. These ulcers recur rapidly and may give the impression of a persistent condition. Herpetiform ulceration is marked by recurrent outbreaks of numerous tiny ulcers—often in the hundreds—ranging from 2 to 3 mm in diameter. These lesions may merge into larger, irregular ulcers and usually heal within 10 to 14 days. Unlike herpetic ulcers, they are not preceded by vesicles and do not contain virally infected cells. Herpetiform aphthous ulcers, which account for approximately

5% of recurrent aphthous stomatitis cases, are more prevalent in women and tend to appear later in life compared to other subtypes. They begin as clusters of small, painful ulcers on an erythematous base, which can coalesce into larger lesions lasting up to two weeks *(Dhanshri et al., 2024).*

Traumatic ulcers are the most frequently encountered form of oral ulceration and are typically acute in presentation. These arise due to physical, thermal, or chemical injuries to the oral mucosa. Common causes include everyday habits such as vigorous tooth brushing or flossing, irritation from sharp dental appliances or teeth, or self-inflicted trauma during dental procedures under local anesthesia. Thermal burns often result from consuming hot foods or beverages—like pizza, coffee, or tea—or from overheated dental instruments used during treatment *(Dhanshri et al., 2024).*



Source: Crispian and Rosemary (2000)

2.2.4 Corneal Ulcer

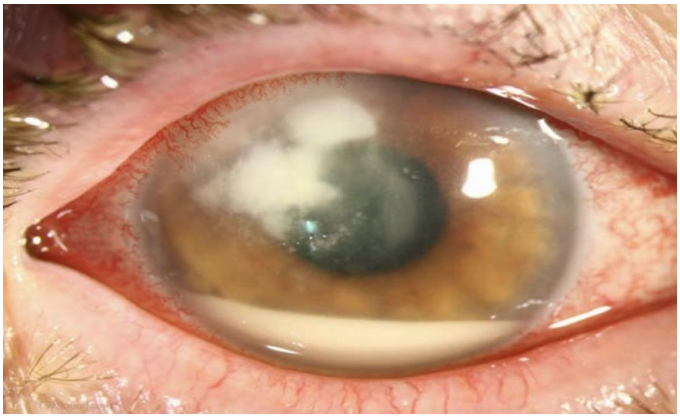
The cornea is the primary refracting surface of the eye, playing a crucial role in focusing light onto the retina. Due to its location on the front of the eye, it is highly susceptible to injuries, infections, and various inflammatory conditions, one of the most serious being corneal ulcers. Defined as a defect in the corneal epithelium accompanied by inflammation, corneal ulcers (also known as keratitis) can significantly impair vision if not promptly treated. While these ulcers can be caused by a variety of factors, the majority are of infectious origin. Bacterial infections are the most common, particularly in individuals who wear contact lenses. However, fungal, viral, and parasitic infections, such as Acanthamoeba keratitis, can also cause corneal ulcers, often in specific environmental or clinical contexts *(Mohan et al., 203)*. Fungal infections, for example, are more frequently seen after agricultural injuries, while Acanthamoeba keratitis primarily affects contact lens users who are exposed to contaminated water. Differentiating between these causes is critical, as the treatment approach varies greatly. Non-infectious causes, though rarer, are also significant. These include neurotrophic ulcers, autoimmune disorders, and exposure keratitis, often resulting from a compromised corneal protective mechanism that leads to persistent epithelial defects *(Mohan et al., 2003)*.

Corneal ulcers are serious conditions that threaten vision, characterized by corneal epithelial defects accompanied by inflammation. They are commonly caused by microbial infections, such as bacterial, viral, fungal, and Acanthamoeba infections, but can also result from mechanical trauma or nutritional deficiencies. If left untreated, these ulcers can lead to severe complications, including corneal perforation and vision loss. Corneal ulcers are generally classified into infectious and non-infectious causes. Infectious causes include microbial agents such as bacteria, fungi, viruses, and Acanthamoeba, while non-infectious causes may

involve trauma, contact lens use, and systemic health conditions. Risk factors for developing corneal ulcers include improper contact lens use or hygiene, and conditions that weaken the corneal surface, such as dry eye or previous ocular surgeries *(Johnson, 2023).*

Corneal ulcers are a leading cause of visual impairment worldwide, with microbial infections and trauma being the primary triggers. A study conducted at the University of Ilorin Teaching Hospital in Nigeria highlighted corneal ulcers as a major contributor to preventable blindness, with microbial keratitis being the most frequent cause *(Adepoju et al., 2023).* This study stressed the importance of early diagnosis and treatment to reduce the visual and economic impacts, especially in low- and middle-income countries, where delayed presentation is often due to reliance on ineffective treatments, such as herbal remedies or over-the-counter drugs *(Adepoju et al., 2023).* In terms of management, a comprehensive review of corneal ulcer treatment emphasizes the need for prompt and appropriate therapy to prevent complications like corneal perforation and vision loss (2023). The World Health Organization (WHO) guidelines also highlight the challenges of managing corneal ulcers in different health systems, underscoring the necessity for standardized treatment protocols (WHO, 2015).

Diagnosis and management of corneal ulcers involve detailed clinical examinations and microbiological testing to identify the causative agent. A study published in the PMC emphasized the importance of a thorough clinical assessment and the use of targeted antimicrobial treatments to improve patient outcomes (PMC, 2007). Additionally, a management guide for general practitioners underscores the vision-threatening nature of corneal ulcers, particularly those caused by trauma or infection (*RACGP, 2022).*



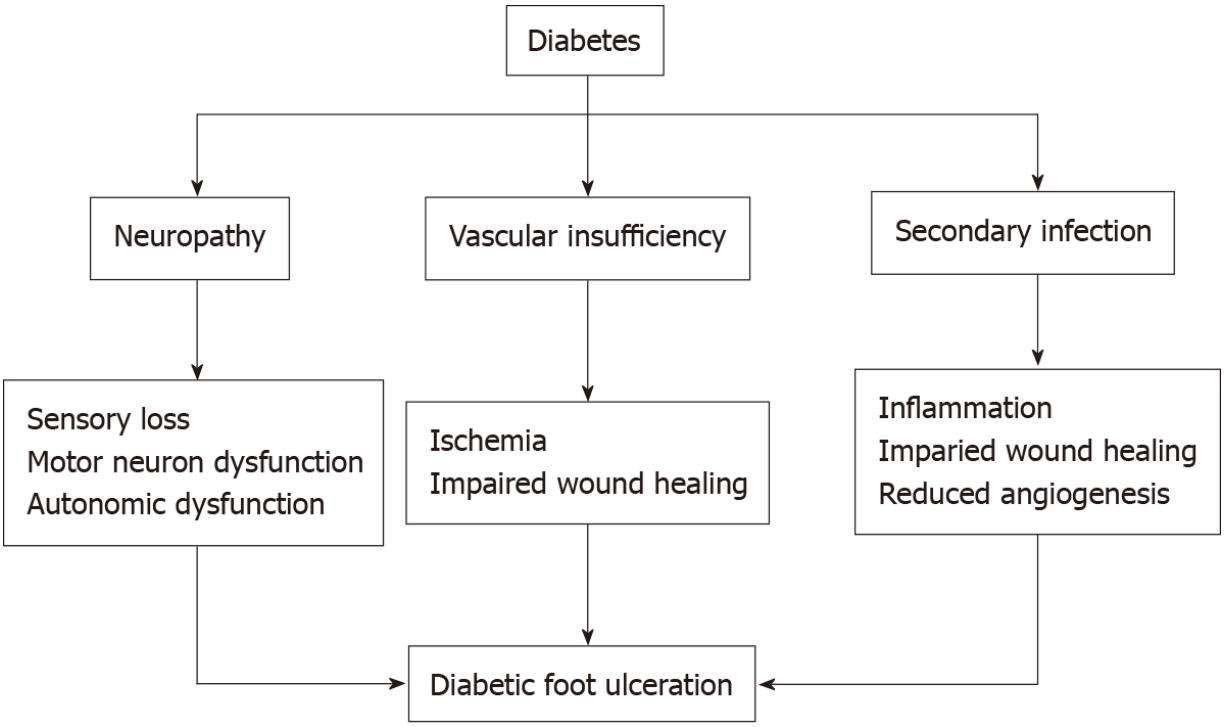
Source: WHO (2015)

2.2.5 Diabetic Ulcer

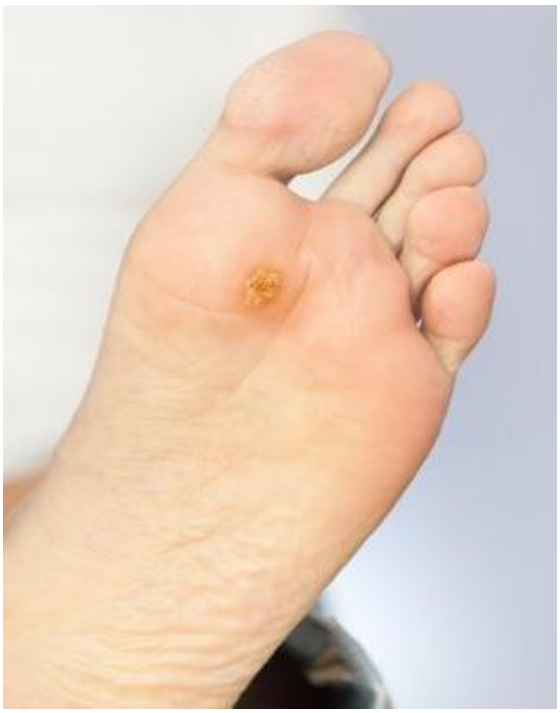
Diabetes mellitus affects approximately 422 million people worldwide and is responsible for an estimated 2 million deaths per year. It affects 11.3% of the United States population *(Raja et al., 2023).* Diabetic ulcer (DU) is a debilitating and severe manifestation of uncontrolled and prolonged diabetes that presents as an ulceration, usually located at the plantar aspect of the foot. Approximately 15% of individuals with diabetes will eventually develop one of these ulcers, and out of these individuals, 14%-24% of them will require amputation of the ulcerated foot due to bone infection or other ulcer-related complications. With such a high level of morbidity stemming from debilitating osteomyelitis and amputation in patients with DU, it is of the utmost importance to properly address and treat the underlying causes of DU. In this paper, we review the current literature with focus on the pathophysiology, preventive options, and definitive management of DU *(Raja et al., 2023).*

Diabetic ulcer comprises a full-thickness wound involving the dermis, located in the weight-bearing or exposed area below the ankle. The Wagner system aids in

categorizing the severity of the ulcer, ranking it on a scale of 1 to 5 (Table 1). The pathologic mechanisms of DFU are described in terms of a triad. This triad includes neuropathy, vascular insufficiency, and secondary infection due to trauma of the foot *(Raja et al., 2023).*



Source: Raja (2023)



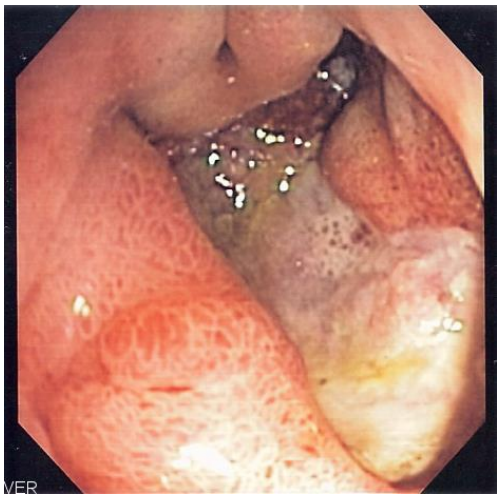
Source: RACGP (2022)

2.2.6 Stomach Ulcer

The process of digestion and the role of the stomach in maintaining health have fascinated humans since ancient times (Modlin, 1995). Stomach ulcers represent a major gastrointestinal issue, affecting millions globally. These ulcers occur when the mucosal lining of the stomach is damaged due to factors such as excessive gastric acid secretion, infection with *Helicobacter pylori*, or prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) *(Sung et al., 2020).* The leading cause of stomach ulcers is *H. pylori* infection, which causes inflammation and weakens the mucosal barrier, making it more vulnerable to damage from stomach acid *(Malfertheiner et al., 2017).* NSAIDs contribute to ulcer formation by inhibiting cyclooxygenase (COX) enzymes, thereby reducing the production of protective prostaglandins and increasing gastric acidity (Lanas and Chan, 2017). Other

contributing factors include smoking, heavy alcohol use, stress, and poor dietary habits *(Sung et al., 2020).*

Several risk factors increase the likelihood of developing stomach ulcers. *H. pylori* infection is the most common, affecting nearly half of the global population *(Malfertheiner et al., 2017)*. Chronic NSAID use, particularly among the elderly, significantly heightens ulcer risk. Smoking and alcohol consumption exacerbate mucosal damage, while chronic stress has been associated with heightened gastric acid secretion and greater susceptibility to ulcers *(Lanas and Chan, 2017).* Research shows that *H. pylori* infection is responsible for the majority of stomach ulcer cases. The bacterium colonizes the gastric epithelium, inducing an inflammatory response that compromises the mucosal barrier, increasing vulnerability to acid-related damage *(Malfertheiner et al., 2017). H. pylori* produces virulence factors such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA), which exacerbate epithelial damage and promote ulcer development *(Sugano et al., 2021).*



Source: Nagm (2011)

2.3 Causes of Ulcer

2.3.1 Psychological Causes

Over time, clinicians have been fascinated by the connections between physical ailments and psychological states. Many researchers have shown that psychological processes can influence the development and progression of medical diseases. Some conditions, once categorized as classic psychosomatic disorders in the 1930s, appear to be directly impacted by psychosocial factors. Peptic ulcer disease (PUD) is considered one of these psychosomatic disorders. Alexander's early psychological theories, based on retrospective data, were expanded upon by the prospective studies of Mirsky and Weiner *(Niasiry and Piper, 1985).*

Recent studies have revealed that peptic duodenal ulcer is a complex condition with varying characteristics. Some patients with duodenal ulcers exhibit elevated or normal pepsinogen levels, which are inherited as an autosomal dominant trait. Approximately two-thirds of duodenal ulcer patients have elevated pepsinogen levels, while the remaining third have normal levels. Elevated serum pepsinogen levels are associated with increased psychopathology, including poor coping abilities, hostility, and hypersensitivity. Other potential genetic markers include blood type O or the absence of ABH blood group antigens in saliva and gastric juice. Despite recognizing the genetic and physiological variability in peptic ulcers, specific psychological traits that may correlate with particular ulcer subgroups have not been clearly defined *(Magni et al., 1987).*

To explore the psychogenic factors in the etiology of peptic ulcer disease, advanced behavioral research methodologies have been employed, leading to three distinct research areas: (1) personality and psychological factors; (2) stressful life events; and (3) biopsychological interactions.

Personality and Psychological Factors: Psychoanalytically oriented researchers have proposed the existence of a unique psychosomatic personality type, distinct from neurotic or psychotic tendencies. This individual tends to be unimaginative, emotionally rigid, introverted, and has difficulty expressing feelings. This person is also more likely to experience psychosomatic disorders. However, this "alexithymia" personality type does not necessarily explain the target organ or disease involved, including peptic ulcer disease. No definitive psychological traits have been consistently linked to peptic ulcers, though these patients often show higher levels of anxiety and depression. Some researchers have suggested that peptic ulcer disease could be a depressive equivalent, though evidence supporting this hypothesis is weak. In a case-controlled study of 49 men with peptic ulcer disease, factors like hypochondriasis, negative life event perceptions, depression, dependency, and lowered ego strength distinguished ulcer patients from controls. Efforts to link type A behavior (commonly associated with coronary artery disease) to peptic ulcer disease have also been made, but it remains unclear whether these psychological findings cause peptic ulcers or are a result of the condition itself.

Stressful Life Events: The impact of stress on the development and progression of physical illnesses, including peptic ulcer disease, has been extensively studied through both animal and human models. The concept of stress, its harmful and beneficial effects, and its controllability have emerged as significant factors in research. Studies have shown that stressful life events correlate with increased physical morbidity. Brady’s “executive monkeys” demonstrated gastric mucosal damage resulting from the stress of decision-making, not just electric shocks. Similarly, Weiss found that immobilized rats, who were unable to control the shocks they received, were more prone to ulcers. Stressful life events, measured by tools such as Holmes and Rahe's Schedule of Recent Events, are associated with greater

morbidity. Other variables, such as a patient's premorbid personality, coping mechanisms, perception of stress, and social support, play crucial roles in determining the physiological response to stress (*Barbara, 1991).*

Biopsychological Interaction: Studies on biopsychological interactions have further emphasized the role of these factors in the development of peptic ulcer disease. Gundry et al. identified two distinct subgroups of duodenal ulcer patients: (1) those with low acid output who tend to be depressed and (2) those with high acid output who tend to be anxious. This finding has been corroborated by other research. Gastrin levels have also been linked to personality traits such as the desire for independence, achievement, and expressiveness. However, research into the relationship between gastric emptying, gastric motility, and psychological factors has been inconclusive. It appears that rapid gastric emptying may be linked to conflicts surrounding issues of dependence and independence *(Barbara et al., 1991).*

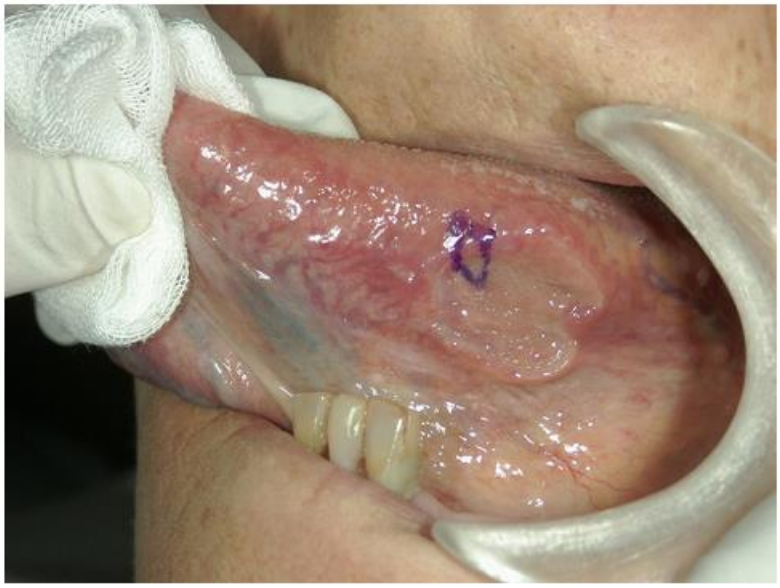
2.3.2 Medication

Ulcers due to drugs are clinically classified into two types. The first is widespread mucositis and ulceration, mainly caused by cytotoxic drugs used for anti-tumor chemotherapy. Widespread sloughing and ulceration arise within days of commencing therapy, with the associated pain often requiring opioid therapy and alteration or cessation of chemotherapy. Such cytotoxic drugs include 5-fluorouracil, methotrexate, bleomycin, and cisplatin. Immunosuppressive agents may also cause oral ulceration through opportunistic secondary infections involving organisms such as Gram-negative bacteria and fungi. The second type is fixed drug eruption, showing repeated development of treatment-resistant ulcers *(Ozkaya, 2013*). Single or multiple large ulcerations are seen on every site of the oral mucosa. Generally, the ulceration is larger than aphthous ulceration, with a flat surface showing slightly

white appear ance. The margin of the ulcer is clear and often slightly raised; however, the ulcers are unaccompanied by any induration. They often resemble traumatic and decubital ulcers, but no irritant factors are apparent in their vicinity. A multiple aphthous ulceration type has also been reported. Topical steroids are ineffective for these forms of ulceration. Histopathological examination usually reveals non-specific ulcer formation with marked infiltration of inflammatory cells. The molecular mechanisms involved with these types of oral ulceration have yet to be clarified, but immunological reactions may play some role in the process *(Field and Allan, 2003).*

Many kinds of drugs cause oral ulcerations, including some b blockers, immunosuppressants, anticholinergic bronchodila tors, platelet aggregation inhibitors, vasodilators, protease inhibitors, antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), antiretrovirals, and antihypertensives (Table 2) *(Scully and Bagan, 2003).* Among these, NSAIDs are popular drugs that are well known to induce oral ulcerations. Several recent reports have described oral ulceration associated with relatively new drugs for the treatment of chronic disorders such as diabetes, angina pectoris, rheumatoid arthritis, and osteo porosis.

Alendronate (bisphosphonate). Alendronate is a drug belonging to the diphosphonate family that has recently been used in the treatment of osteoporosis and other bone diseases. This drug has been demonstrated to induce progressive and significant increases in bone mineral density in women with osteoporosis. Bisphosphonate-related osteonecrosis of the jaw is a well-established adverse effect of bisphosphonates, but oral ulceration as a result of taking alendronate has also recently been reported. These oral ulcerations are induced by incorrect use of the drugs and are caused by the drugs causing direct irritation Jinbu and Demitsu, (2014).



Source: Jinbu and Demitsu (2014)

The use of dopamine and the use of corticosteroids8 have been reported as risk factors for the development of pressure ulcer. Few reports have investigated the effect of prescription medications on pressure ulcer after it has developed. In addition, certain medications have been reported to delay wound healing, but the effect of medications on pressure ulcer remains unclear. Non-steroidal anti-inflammatory drugs (NSAIDs) ongoing use of this class of medications is the second most common cause of ulcers *(Arai et al., 2020).*These drugs (which include Indomethacin, ibuprofen, naproxen, diclofenac, tolmetin, piroxicam, fenoprofen, indomethacin, oxaprozin, ketoprofen, sulindac, nabumetone, etodolac, and salsalate) are acidic. They block prostaglandins, substances in the stomach that help maintain blood flow and protect the area from injury. Some of the specific drugs listed are more likely to produce ulcers than others. Therefore, if you must use long-term pain medications, talk to your doctor about which ones are safest *(Debjit et al., 2010).*

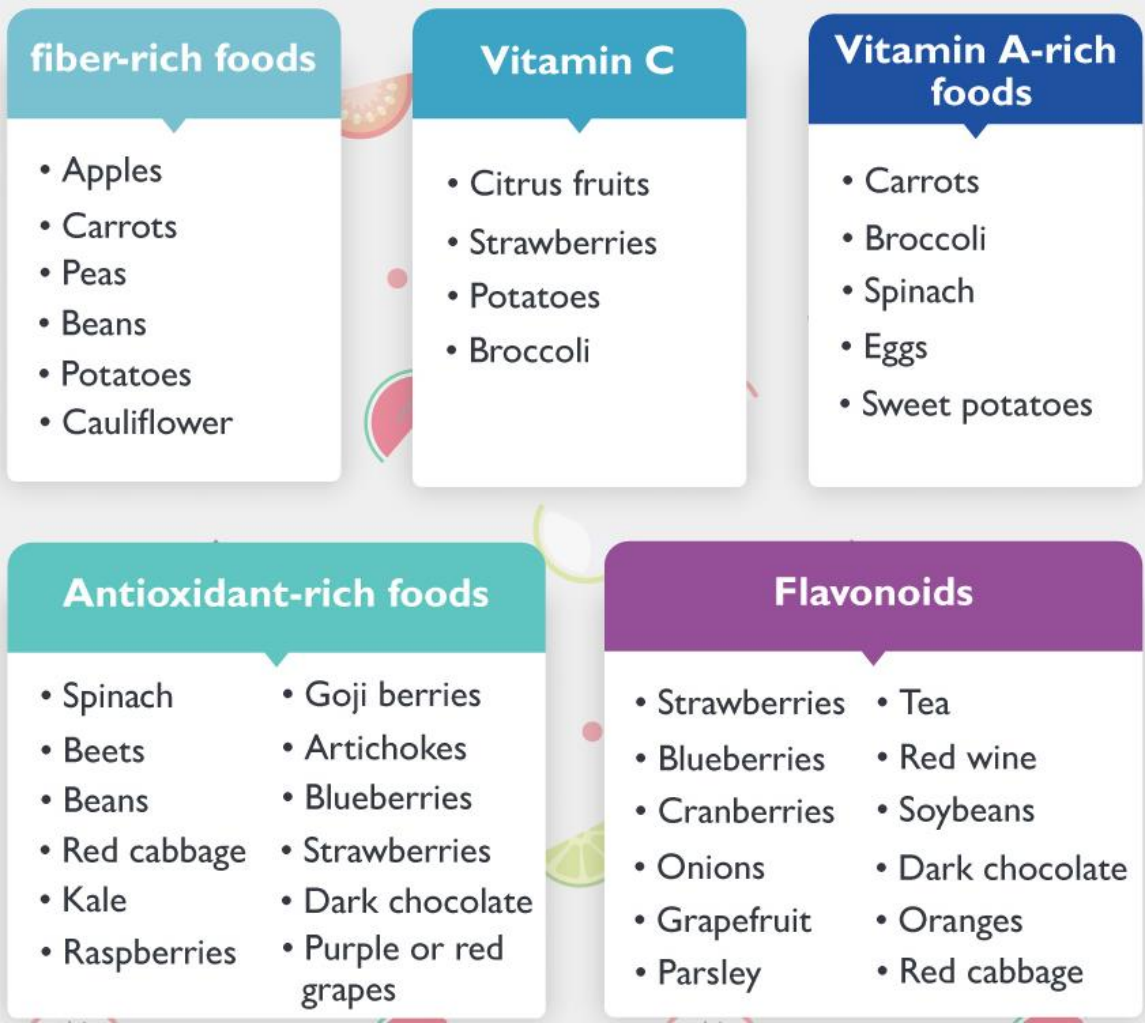
2.3.3 Dietary Factors

Various factors contribute to ulcer development, including Helicobacter pylori infection, nonsteroidal anti-inflammatory drug (NSAID) use, and lifestyle factors such as diet (Lanas and Chan, 2017). Spicy foods, particularly those containing capsaicin, have been controversially linked to ulcer development. Some studies suggest that excessive consumption of spicy foods can exacerbate gastric mucosal irritation and increase acid secretion (Zhu *et al*., 2019). However, other research indicates that capsaicin may have protective effects by stimulating mucus secretion and promoting gastric mucosal blood flow (Satyanarayana *et al*., 2021). Despite these conflicting findings, individuals with existing ulcers are often advised to limit spicy food intake.

A diet high in sodium has been implicated in gastric mucosal damage and increased susceptibility to H. pylori infection, which is a major risk factor for ulcers (Huang *et al*., 2017). High salt intake may alter gastric mucus composition and impair its protective functions, thereby making the stomach lining more vulnerable to acid-induced injury *(Gaddy et al., 2019).* Excessive alcohol consumption has been shown to contribute to ulcer formation by weakening the gastric mucosal barrier and increasing acid production (Jiang *et al*., 2021). Alcohol can also stimulate inflammatory responses and delay ulcer healing by interfering with prostaglandin synthesis *(Matsuhashi et al., 2018).* Chronic alcohol consumption is particularly associated with a higher risk of gastric ulcers. Caffeinated and acidic beverages, including coffee, tea, and carbonated drinks, have been linked to increased gastric acid secretion, which can exacerbate ulcer formation (Yuan *et al*., 2020). While caffeine alone does not directly cause ulcers, it may worsen symptoms in individuals predisposed to gastric irritation (Maldonado *et al*., 2021). Acidic drinks, such as

citrus juices, may also irritate the gastric lining, further contributing to mucosal damage.

Diets rich in processed foods containing artificial additives, preservatives, and high levels of unhealthy fats have been associated with increased ulcer risk *(Zhang et al., 2019).* These foods may promote inflammation and oxidative stress, which can weaken the mucosal defense system and contribute to ulcerogenesis *(Patel and Shah, 2022).* While certain dietary habits contribute to ulcer formation, others may offer protection. Diets rich in fiber, particularly from fruits, vegetables, and whole grains, have been shown to reduce ulcer risk by promoting healthy gut microbiota and enhancing mucosal integrity *(Song et al., 2018*). Additionally, flavonoid-rich foods such as apples, onions, and green tea have demonstrated gastroprotective properties (Liu *et al*., 2020).



Source: (www.sprintmedical.in)

2.4 Pathophysiology of Ulcer

In recent years the hospitalization rate for duodenal ulcer (DU) has decreased by 43%, that for gastric ulcer (GU) by 8% and mortality by ca. 62%. At first sight these figures might suggest that the disease as such is dying out. This is an illusion based to some extent on improvements in diagnosis, therapy and control of complications. Gastric acid (HCl) and pepsin play a crucial role in digestion but can also contribute to mucosal injury when unregulated. The parietal cells of the stomach secrete acid under the influence of histamine, gastrin, and acetylcholine *(Kumar et*

al., 2021). Excessive acid production leads to mucosal erosion and ulcer formation, especially in conditions such as Zollinger-Ellison syndrome (Sundaram and Sitaraman, 2018). Pepsin, a proteolytic enzyme, further degrades the mucosal proteins, worsening ulceration *(Wang et al., 2019).* Direct incidence studies in the USA and Denmark show that ulcer occurrence is unchanged, i.e., 10% of men and ca. 5% of women in the Caucasian western population are expected to have some form of ulcer disease during their lifetime. This unchanged trend is genetically programmed: 30- 59% of ulcer patients have a positive family history in contrast to 5-15% of control persons. DU and GU follow separate genetic pathways *(Holle, 2010).*

What are the changes brought about by stimulation that protect the gastric mucosa? It normally activates epithelial mucus and bicarbonate production. This is pronounced in ulcer patients, particularly in gastroduedenal (GD) type. However, in these patients the genetically induced changes in the gastrointestinal motility and also the alterations in the chemical composition of the mucus with the increased low molecular glycoproteins are pathological, as proteolytic decomposition from the lumen and H+-ion rediffusion are favored. Stimulation normally influences gastric blood flow. It is known that no necrosis develops when there is a 4-fold increase in flow or when the mucous membrane is perfused with pure O2. In comparison, when the mucosal blood flow is reduced subliminal noxae cause an increase in H+-ion rediffusion and extreme damage to the mucosa. There is, however, no ulcer without acid. In DU and GU the lower limit of acid stimulation is 10 mEq/h. Some 50-70% of DU are hyperacidic with a 50% increase in the maximal acid output MAO. The cephalic phase also brings an increase in secretion response of 50-70% (Holle, 2010).

The majority of the Helicobacter pylori bacteria live freely in colonized hosts, but ~20% are believed to bind gastric epithelial cells. The bacteria colonize the gastric mucosa, producing urease, which converts urea into ammonia, neutralizing stomach acid and allowing bacterial survival *(Hooi et al., 2017).* Additionally, H. pylori releases cytotoxins such as CagA (cytotoxin-associated gene A) and VacA (vacuolating cytotoxin A), leading to epithelial cell damage, inflammation, and disruption of gastric mucosal integrity (Chey and Leontiadis, 2018).

This colonization is highly specific in vivo when it overlays islands of gastric metaplasia *(Peek and Crabtree, 2006).* The interaction of these bacteria with the epithelial cells plays an important role in the pathogenesis of cancer risk. Loci have been identified the mucosa in which people harboring particular alleles which have different risk of disease, and Helicobacter pylori should have the capacity to interact with those molecules that induce epithelial response with carcinogenic potential. Helicobacter pylori is well characterized as determining the 'cag' pathogenicity island (cag PAI), a multigene locus. It induces gastritis augmenting the risk for atrophie gastritis and distal gastric cancer. In most people, however, it remains asymptomatic. It would be useful to identify a few people with high risk, because they could serve as a paradigm for the chronie role of inflammation in the genesis of malignancies that arise in the gastrointestinal tract. Helicobacter pylori is not the only organism that is believed to cause active chronie gastritis in man. Other infections with spiral organisms have been described (Israel and Peek, 2006).

The bacillus is sensitive to penicillin, erythromycin, cephalosporins, gentamycin, tetracycline and bismuth citrate. Ranitidine has been combined with bismuth citrate (RBC), clarithromycin or amoxycillin. In 70% of patients the peptic ulcer healed within 4 weeks, but only 41-48% of the Helicobacter organism were eradicated. In a randomized study of 900 patients with peptic complaints an average

of 75% of those given the combination of omeprazole plus clarithromycin, had Helicobacter eradication, but at 6 months 30% in one study and 52% in another had ulcer recurrence. In a third study at final analyses after 4-6 weeks 50-70% of ulcers had not healed (*Bamett, 1995).*

NSAIDs, such as Indomethacin and ibuprofen, contribute to ulcer formation by inhibiting cyclooxygenase (COX) enzymes, specifically COX-1, which is responsible for prostaglandin synthesis *(Cryer and Mahaffey, 2019).* Prostaglandins are essential for maintaining gastric mucosal protection by stimulating mucus and bicarbonate secretion and promoting blood flow. The inhibition of COX-1 leads to increased gastric acid production and reduced mucosal defense, making the stomach lining more susceptible to damage *(Lanza et al., 2016).* The gastric mucosa is protected by a barrier system composed of mucus, bicarbonate, and epithelial integrity. Mucus acts as a physical barrier, while bicarbonate neutralizes gastric acid at the epithelial surface *(Allen and Flemström, 2018).* When these protective mechanisms are compromised due to oxidative stress, ischemia, or chronic inflammation, the mucosa becomes vulnerable to acid-induced injury (*Wallace et al., 2020).* Oxidative stress plays a key role in ulcer pathogenesis by increasing reactive oxygen species (ROS), which damage lipids, proteins, and DNA in gastric cells *(Bhattacharyya et al., 2018).* Pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), further contribute to mucosal damage by inducing apoptosis and impairing tissue repair (Sostres *et al*., 2017). Duodenal ulcers are primarily caused by *H. pylori* infection and excessive acid secretion. Unlike gastric ulcers, which are often linked to impaired mucosal protection, duodenal ulcers occur due to increased gastrin production, leading to elevated acid levels that overwhelm bicarbonate buffering in the duodenum (Feldman and Graham, 2021). Gastric metaplasia, where gastric-type epithelial cells

appear in the duodenum, provides a niche for *H. pylori* colonization, further exacerbating ulceration *(Malfertheiner et al., 2018).*

2.5 Antioxidants

Antioxidants are molecules that inhibit the oxidation of other molecules, thereby preventing the formation of free radicals highly reactive and unstable atoms that can damage cells, proteins, and DNA. Oxidative stress, resulting from an imbalance between free radicals and antioxidants in the body, has been linked to a variety of chronic diseases, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders like Alzheimer's disease *(Pham-Huy et al., 2008).* The human body naturally produces some antioxidants, such as glutathione, but many are also obtained from dietary sources. Common dietary antioxidants include vitamins C and E, beta-carotene, selenium, and various polyphenols found in fruits, vegetables, nuts, and whole grains. These substances help neutralize free radicals and reduce oxidative damage, thereby playing a protective role in maintaining health and slowing the aging process (*Lobo et al., 2010).*

Antioxidants function through several mechanisms, such as donating electrons to stabilize free radicals or chelating metal ions that catalyze free radical production. Additionally, some antioxidants can stimulate the body's own antioxidant defenses by activating genes involved in protective responses (Halliwell and Gutteridge, 2015). In recent years, the role of antioxidants in disease prevention has gained considerable attention. Although antioxidant supplements are widely used, evidence suggests that consuming antioxidants through whole foods is more effective and safer than relying on synthetic supplements. Thus, maintaining a diet rich in natural antioxidant sources is recommended for overall health and disease prevention *(Lobo et al., 2010).*

2.6 Types of antioxidant

Antioxidants are broadly classified into enzymatic and non-enzymatic categories based on their mechanism of action and origin. Both types are essential in maintaining redox homeostasis by neutralizing free radicals and preventing oxidative damage to cells and tissues.

2.6.1 Non enzymatic antioxidant

Non-enzymatic antioxidants are small molecules—either synthesized by the body or acquired through diet—that protect cells by directly scavenging free radicals and neutralizing reactive oxygen species (ROS). Unlike enzymatic antioxidants that catalyze reactions, non-enzymatic antioxidants act by donating electrons or hydrogen atoms to stabilize radicals without becoming reactive themselves. Common non-enzymatic antioxidants include vitamin C (ascorbic acid), vitamin E (tocopherol), glutathione, carotenoids, polyphenols, and flavonoids. Vitamin C is water-soluble and functions primarily in the aqueous compartments of cells, such as cytoplasm and plasma, where it neutralizes hydroxyl and superoxide radicals. Vitamin E, a lipid-soluble antioxidant, protects cell membranes from lipid peroxidation by reacting with lipid radicals and terminating chain reactions (*Pisoschi and Pop, 2015).*

2.6.1.1 Total phenolic content

Non-enzymatic antioxidants play a vital role in protecting biological systems from oxidative stress, which arises when there's an imbalance between the production of free radicals and the body’s ability to detoxify them. Among the most important non-enzymatic antioxidants are phenolic compounds, which are naturally found in plants and known for their strong radical-scavenging abilities. The

cumulative measure of these compounds in a sample is referred to as the Total Phenolic Content (TPC), and it is a widely accepted indicator of a plant's antioxidant potential. In recent years, medicinal plants like *Sida acuta* have gained attention for their high TPC and associated health benefits. TPC is typically assessed using the Folin–Ciocalteu assay, and results are expressed in gallic acid equivalents (GAE). A high TPC in *Sida acuta* signifies a rich presence of bioactive phytochemicals such as flavonoids, tannins, and phenolic acids, which contribute to its therapeutic properties, including anti-ulcer, anti-inflammatory, and antioxidant effects (Ogunmoyole *et al*., 2015). Phenolic compounds work by directly scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby preventing the oxidative damage of cell membranes, proteins, and DNA. In the context of peptic ulcer disease, oxidative stress plays a major role in gastric mucosal injury, often triggered by factors such as *Helicobacter pylori* infection, NSAID use, or ethanol-induced gastric irritation. The phenolic-rich extracts of *Sida acuta* have been shown to mitigate such damage by reducing lipid peroxidation and enhancing mucosal protection (Akinmoladun *et al*., 2020). In animal studies, *Sida acuta* extracts significantly reduced gastric ulceration scores and improved antioxidant enzyme levels, such as glutathione and superoxide dismutase, which are often suppressed in oxidative conditions. These outcomes were strongly linked to the plant's non-enzymatic antioxidant constituents, particularly its total phenolic content *(Ezekwesili et al., 2014).*

2.6.1.2 Total flavonoid content

Flavonoids are a large class of naturally occurring polyphenolic compounds that serve as powerful non-enzymatic antioxidants. They are known for their capacity to scavenge free radicals, inhibit oxidative damage, and modulate inflammatory responses, making them central to the prevention and management of

numerous health conditions, including peptic ulcer disease. The Total Flavonoid Content (TFC) of a plant extract is a quantifiable measure of its flavonoid richness, often used as a marker of its antioxidant potential. In medicinal plants like *Sida acuta*, TFC plays a crucial role in its gastroprotective, anti-inflammatory, and wound-healing properties. TFC is typically determined using the aluminium chloride colorimetric method and results are expressed in quercetin equivalents (QE). Studies on *Sida acuta* have revealed that it contains a high flavonoid content, particularly in its ethanolic and aqueous leaf extracts, which correlates with its notable antioxidant activity (*Ogunmoyole et al., 2015).*

Flavonoids in *Sida acuta* contribute to ulcer prevention through several biological mechanisms. Firstly, they neutralise reactive oxygen species (ROS) and reduce lipid peroxidation, which is a major cause of gastric mucosal damage in ulcer formation. Secondly, flavonoids enhance gastric mucosal defense by stimulating prostaglandin synthesis and increasing mucus secretion, both of which are essential for protecting the stomach lining from irritants such as ethanol, NSAIDs, or *Helicobacter pylori (Ezekwesili et al., 2014).* Furthermore, flavonoids are also known to inhibit histamine release and modulate cytokine production, thereby reducing inflammation and promoting tissue repair. These properties make them vital components in the healing of existing ulcers and the prevention of new ones. In ulcer models, *Sida acuta* extracts rich in flavonoids have demonstrated a significant reduction in ulcer index, suggesting that TFC directly contributes to the plant's anti-ulcer efficacy (***Akinmoladun et al., 2020).***

2.6.1.3 Ferric reducing antioxidant power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay is a well-established analytical method used to evaluate the antioxidant potential of plant extracts. It

measures the ability of a sample to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) under acidic conditions. The higher the reducing capacity, the stronger the antioxidant activity, which is crucial in preventing oxidative damage implicated in several pathological conditions, particularly peptic ulcers. In this context, *Sida acuta*, a medicinal plant long used in traditional systems for treating stomach-related disorders, has demonstrated notable FRAP activity, especially in its leaf extracts. The antioxidant power exhibited is primarily attributed to its abundance of polyphenolic compounds, especially flavonoids and phenolic acids, which are known for their electron-donating capacity (*Sharma et al., 2022).* Oxidative stress plays a central role in ulcerogenesis, contributing to mucosal erosion through the generation of free radicals that damage cell membranes, proteins, and DNA. Substances with high FRAP values like *Sida acuta* can interrupt this process by donating electrons to unstable radicals, thus halting their damaging cascade. Such antioxidant activity enhances gastric mucosal protection and supports healing of existing lesions (Mehta *et al*., 2021). Additionally, the FRAP assay reflects the total antioxidant effect of all compounds present in the extract. In *Sida acuta*, these include flavonoids, tannins, and saponins, which may act synergistically to neutralise free radicals and reduce inflammation. This synergistic action contributes to the plant's broader therapeutic roles, including its gastroprotective and anti-inflammatory effects (*Kumar et al., 2020).*

2.6.1.4 2,2-azino-bis C3 ethylbenzothiazoline-b-sulphonic acid (ABTS)

The ABTS [2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] assay is a reliable method for determining the antioxidant capacity of natural compounds and plant extracts. It evaluates the ability of antioxidants to quench the $ABTS^{+}$• radical cation, producing a measurable reduction in absorbance. The higher the antioxidant activity, the more effectively the extract neutralises free radicals, making this assay

especially relevant in studies exploring the gastro protective potential of medicinal plants like *Sida acuta*. *Sida acuta*, a plant traditionally used to treat inflammation and gastrointestinal discomfort, has been shown to possess strong ABTS radical scavenging activity, particularly in its metabolic and aqueous extracts *(Adepoju et al., 2021).* This activity is directly linked to the presence of flavonoids, tannins, and polyphenolic compounds, which are known to donate hydrogen atoms or electrons to stabilise free radicals and prevent cellular damage.

The antioxidant effect measured by the ABTS assay is important in the context of peptic ulcer disease, as oxidative stress plays a significant role in the breakdown of gastric mucosa. Excessive generation of reactive oxygen species (ROS) during stress, NSAID use, or *Helicobacter pylori* infection can lead to mucosal injury. The ability of *Sida acuta* extracts to scavenge ABTS radicals suggests that the plant can protect gastric tissue by neutralising free radicals, thereby preventing or reducing ulcer formation *(Nwanya et al., 2020).* Furthermore, unlike some assays that measure only lipid peroxidation or ferric reduction, the ABTS assay provides a broad-spectrum measurement of antioxidant capacity, effective in both hydrophilic and lipophilic systems. This makes it especially valuable in evaluating complex plant matrices like *Sida acuta*, which contains a variety of antioxidant compounds that may act synergistically *(Odeyemi et al., 2022)*

2.6.1.5 2,2-diphenyl-1-pycrylhydraxyl (DPPH)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a widely used and simple method for evaluating the free radical scavenging activity of plant extracts. It measures the ability of antioxidants in a sample to reduce the DPPH radical, a stable purple-colored compound, into a yellow-colored non-radical form. The reduction in absorbance is directly proportional to the antioxidant strength of the sample, offering

valuable insight into its potential biological protective effects. Medicinal plants like *Sida acuta*, which are traditionally used for treating inflammation, wounds, and stomach disorders, have shown strong DPPH radical scavenging activity. This suggests that the plant is a rich source of non-enzymatic antioxidants, particularly phenolic compounds and flavonoids, which are known for their ability to donate electrons or hydrogen atoms to neutralise free radicals *(Olasehinde et al., 2022).*

In the context of peptic ulcer disease, oxidative stress is one of the main culprits in mucosal injury, particularly when triggered by ethanol, NSAIDs, or *Helicobacter pylori*. Antioxidants that effectively quench radicals like DPPH can significantly mitigate gastric epithelial cell damage, promote tissue repair, and improve mucosal defense mechanisms. Extracts of *Sida acuta* with high DPPH scavenging capacity have been found to reduce oxidative stress markers, thereby supporting their gastroprotective potential *(Iroanya and Onajobi, 2020).* Compared to other assays, DPPH is particularly useful for evaluating lipophilic antioxidants, making it an ideal method for investigating the total antioxidant capacity of plant-based remedies. In the case of *Sida acuta*, DPPH activity correlates well with its high total phenolic and flavonoid content, further confirming its role in protecting the gastrointestinal tract against oxidative damage *(Ajiboye et al., 2021).*

2.6.2 Enzymatic antioxidant

Enzymatic antioxidants are endogenous proteins that protect the body from oxidative damage by catalyzing reactions that neutralize reactive oxygen species (ROS). These enzymes are a primary line of defense and play a crucial role in preventing cellular damage caused by free radicals. The major enzymatic antioxidants include superoxide dismutase (SOD)**,** catalase (CAT)**,** and glutathione peroxidase (GPx)**.** SOD is responsible for converting the highly reactive superoxide

anion (O_2^-) into hydrogen peroxide (H_2O_2), a less reactive compound. Catalase then decomposes hydrogen peroxide into water and oxygen, preventing its accumulation and subsequent conversion into harmful hydroxyl radicals. Glutathione peroxidase also reduces hydrogen peroxide and lipid peroxides, using glutathione as a substrate, thereby playing a vital role in maintaining membrane integrity and cellular function *(Apel and Hirt, 2004).*

These enzymatic antioxidants are particularly important in organs with high metabolic activity, such as the brain and liver, where oxidative metabolism generates substantial ROS. Their activity is tightly regulated and can be induced by oxidative stress, inflammation, and certain dietary components. Unlike non-enzymatic antioxidants that scavenge free radicals directly, enzymatic antioxidants work through multi-step processes and offer continuous protection. Their efficiency and specificity make them essential for cellular defense and survival, particularly under stress conditions (Mittler, 2002)

2.6.2.1 Superoxide dismutase

Superoxide dismutase (SOD) is among the most potent antioxidants known in nature and is an important constituent of cellular defense against oxidative stress. The enzyme shows several interesting properties like very high catalytic rate of reaction and high stability to physiochemical stress. It has also attracted widespread interest due to its therapeutic potential. Oxidative stress is known to be involved in pathophysiology of several diseases and SOD supplementation has been shown to be beneficial in treatment or prevention of such diseases *(Bafana et al., 2011)*

SOD is ubiquitous to all forms of life. Four different types of metal centers have been detected in SOD, dividing this family into Cu,Zn-, Fe-, Mn- and Ni-SODs.

The evolution of SOD and other antioxidant enzymes was probably triggered by production of O2 by photosynthetic organisms about 2 billion years ago. Two major kinds of SOD appeared independently in prokaryotes at that time, Cu, Zn SODs and Fe SODs/Mn SODs. Fe/Mn SODs then evolved into Fe and Mn SODs by gene duplication. This may be the reason why Fe and Mn SODs are closely related with regard to three-dimensional structure and amino acid sequence. However, their crystal structures and catalytic mechanism are completely different as compared to Cu, Zn SOD, supporting the hypothesis of independent evolution *(Shin et al., 2009).*

In the Fe- and Mn SOD group, Fe SOD is proposed to be more ancient because of an abundance of Fe in soluble Fe(II) form on primitive earth. As the level of O2 in the primitive environment increased, availability of Fe(II) decreased, probably causing a shift to the use of more available Mn. A phylogenetic tree of Fe- and Mn SODs shows short distances separating Fe SODs from Mn SODs, confirming a common phylogenetic origin for these two, and suggesting likely frequent horizontal gene transfer. The tree also clearly separates archaeal SODs from other prokaryotic SODs (Schafer and Kardinahl, 2003).

The catalytic mechanism of SOD is described by the following reaction sequence:

$$M^{3+} + O_2^{\bullet} + H^+ \rightarrow M^{2+}(H^+) + O_2$$

$$M^{2+}(H^+) + H^+ + O_2^{\bullet} \rightarrow M^{3+} + H_2O_2$$

where, M stands for metallic cofactor. This stepwise mechanism confers several advantages to the reaction thermodynamics. Firstly, potential electrostatic repulsion between two O_2 • anions is overcome by reacting with only one molecule at a time. Specific binding to negatively charged O_2 is mediated by the positively charged

metals in the active site. In the second step with reduced metal ion, active site's electrostatic attraction is preserved by the uptake of a proton *(Bafana et al., 2011)*

2.6.2.2 Catalase

Catalase is a vital antioxidant enzyme present in nearly all aerobic organisms, where it plays a key role in cellular defense against oxidative stress. Its primary function is to decompose hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), into water and molecular oxygen, thus preventing the accumulation of H_2O_2 and subsequent oxidative damage (Chelikani, Fita, and Loewen, 2004). Hydrogen peroxide is produced as a by-product of various metabolic processes, and if not efficiently removed, it can participate in the Fenton reaction to generate hydroxyl radicals, which are highly reactive and capable of initiating lipid peroxidation *(Halliwell and Gutteridge, 2015).*

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Catalase plays a protective role by limiting the availability of H_2O_2, thereby reducing the potential for hydroxyl radical formation and subsequent lipid peroxidation. In tissues with high catalase activity, ROS are more effectively neutralized, resulting in lower levels of lipid peroxidation products. Conversely, a decrease in catalase activity can lead to elevated oxidative stress and increased lipid peroxidation, contributing to the pathogenesis of several diseases, including neurodegenerative disorders, cardiovascular diseases, diabetes, and cancer *(Zhang et al., 2016).*

2.7 Lipid peroxidation parameters

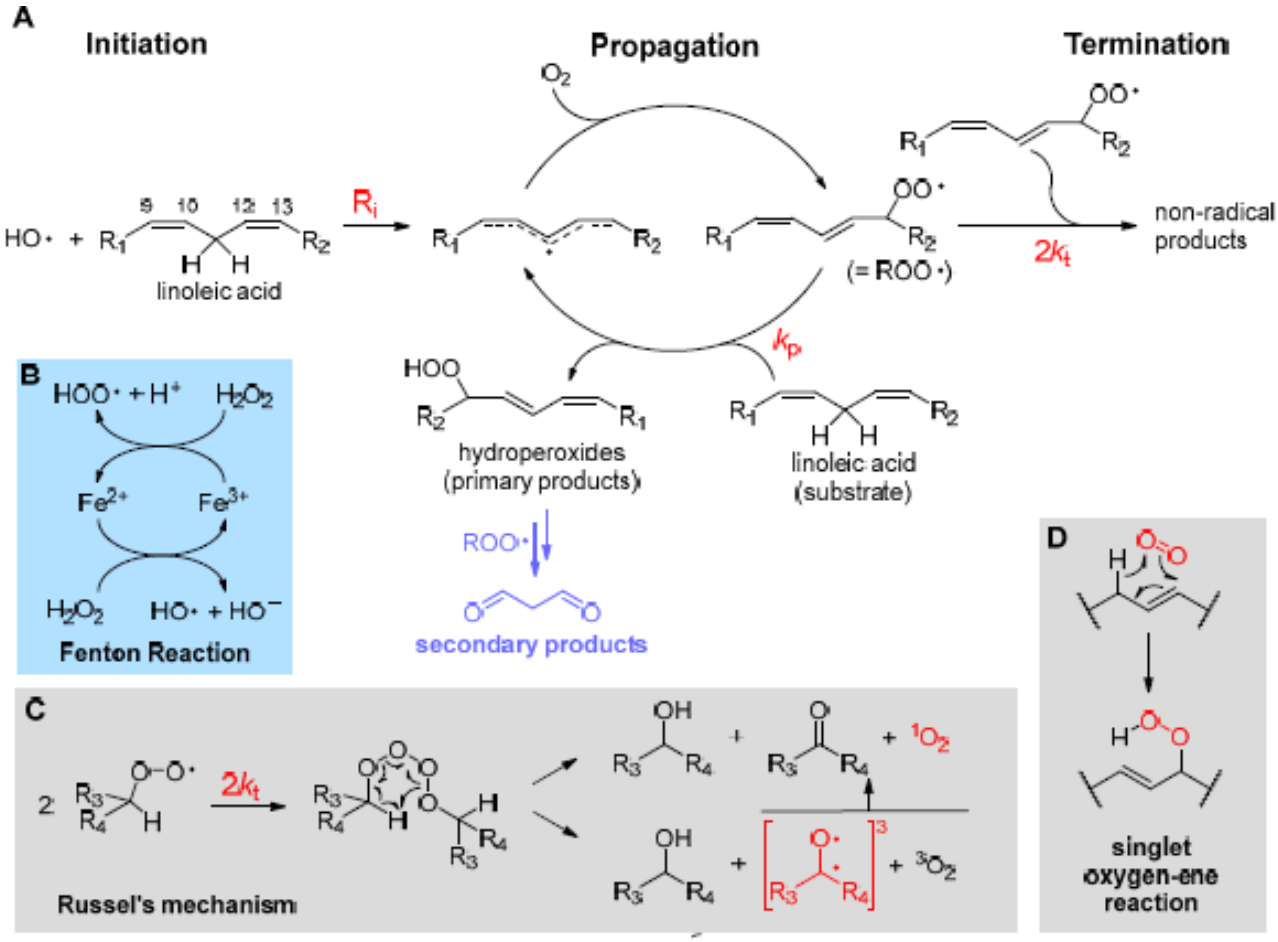
Antioxidants are a very heterogeneous class of compounds, small molecules and enzymes that share the task of protecting oxidizable molecules or materials from oxidative transformation. In the biological context, the reference oxidative process is lipid peroxidation (LP); therefore, antioxidants are typically defined and discussed on the basis of their ability to prevent, slow down or block LP *(Valgimigli et al., 2012).* Based on where and how they interfere with the LP radical chain, antioxidants are classified as preventive, if they impair the initiation process, and chain-breaking if they block or slow down the propagation, while a new category, the termination-enhancing antioxidants, was recently introduced by our group, to include those molecules, such as some terpenes and terpenoids from essential oils, which act by favoring the radical-chain termination without actually impairing propagation *(Amorati and Valgimigli, 2018)*

Lipid peroxidation (LP) is a complex phenomenon, first investigated in the early 20^{th} century, consisting in the uptake of molecular oxygen by lipids exposed to air, which was soon recognized as bearing remarkable similarity to hydrocarbon autoxidation, the formal insertion of one molecule of oxygen in the C-H bond of a hydrocarbon to afford a hydroperoxide:

$$R\text{-}H + O_2 \longrightarrow R\text{-}OO\text{-}H.$$

Indeed, lipid peroxidation is one embodiment of hydrocarbon autoxidation. While the direct reaction with ground state (triplet) oxygen is spin restricted and too slow to occur, the transformation of hydrocarbons (or lipids) by oxygen to hydroperoxides and further oxidized products occurs rapidly and efficiently via the intermediation of peroxyl radicals (ROO_), in a chain reaction that can be triggered by a multitude of events in any chemical system, such as in food or in living

organisms, and it can be blocked or prevented by antioxidants. LP is a radical chain reaction composed of the canonical three stages of initiation,
propagation and termination, summarized in Figure 2.11 using PUFA as the prototypical oxidizable substrate (Valgimigli, 2023).



Source: Valgimigli, (2023)

2.7.1 Glutathione transferase

Glutathione (GSH) is a low molecular weight compound composed of three amino acids: glycine, cysteine and glutamic acid. GSH is present in all plant and animal cells. In physiological conditions it is synthesized in many different tissues *(Forman et al., 2009)*, but the most intense GSH synthesis occurs in hepatocytes (Lu, 2013). Glutathione in the human body is present in several redox forms, among

which the most important are reduced glutathione (GSH) and oxidized glutathione (GSSG).

Blood plasma, for example, contains only about 20mM of glutathione and the dominant form there is oxidized glutathione (GSSG) *(Lushchak et al., 2012).* The concentration and role of GSH are differentiated and cell type-specific. Besides being a potent antioxidant, GSH has a number of functions not related to defence against ROS. For example, it participates in the detoxification processes of electrophilic compounds (xenobiotics), and in the metabolism of prostaglandins and leukotrienes. It is also involved in the transport of amino acids and in the absorption of micronutrients from the intestine, mainly iron and selenium. However, the predominant role of GSH is undoubtedly that of antioxidant. GSH as an antioxidant participates in several lines of defence against ROS. It plays an important role not only as a free radical scavenger, but is also engaged in the repair processes of damaged cells *(Mirończuk-Chodakowska et al., 2018)*

Glutathione transferases (GSTs) are a family of multifunctional enzymes that play a central role in cellular detoxification and antioxidant defense. These enzymes catalyze the conjugation of reduced glutathione (GSH) to a wide range of electrophilic and hydrophobic compounds, including products of oxidative stress such as lipid peroxides, xenobiotics, and environmental toxins (Hayes, Flanagan, and Jowsey, 2005). By facilitating the conjugation of GSH, GSTs help increase the solubility of toxic compounds, making them easier to excrete from the cell. This function is crucial for maintaining redox homeostasis and protecting cellular macromolecules from damage.

In addition to their detoxification role, GSTs are also involved in the modulation of signaling pathways related to cell proliferation, apoptosis, and stress

responses *(Townsend and Tew, 2003*). For example, some isoforms of GST can regulate the activity of kinases and other proteins through interactions that do not involve their catalytic function. Furthermore, GSTs can bind and sequester reactive lipid peroxidation products such as 4-hydroxynonenal (4-HNE), which are toxic and capable of forming adducts with proteins and DNA *(Singhal et al., 2015).* GST activity is often used as a biomarker of oxidative stress and cellular response to toxic insult. Elevated or suppressed GST activity has been reported in various pathological conditions, including cancer, neurodegenerative diseases, and cardiovascular disorders (Board and Menon, 2013). Moreover, polymorphisms in GST genes can affect individual susceptibility to environmental toxins and disease risk, highlighting their importance in toxicology and pharmacogenomics (Board and Menon, 2013).

2.7.2 Glutathione peroxidase

Glutathione peroxidase (GPx) is a family of selenium-dependent antioxidant enzymes that play a critical role in protecting cells from oxidative damage. These enzymes catalyze the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides into water and corresponding alcohols using reduced glutathione (GSH) as a substrate, thereby preventing the formation of reactive oxygen species (ROS)-induced damage (Brigelius-Flohé and Maiorino, 2013). The general reaction catalyzed by GPx is as follows:

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

This process helps maintain redox balance in cells by detoxifying harmful peroxides and regenerating reduced forms of critical molecules. Among the GPx isoforms, GPx1 is the most abundant and ubiquitously expressed in the cytoplasm of nearly all mammalian tissues, while other isoforms such as GPx4 are involved in

reducing lipid hydroperoxides within membranes and lipoproteins (Lubos, Loscalzo, and Handy, 2011). GPx4, in particular, is crucial for protecting against lipid peroxidation and is essential for embryonic development and cellular viability.

GPx activity is a key marker of the antioxidant capacity of cells and tissues. Decreased GPx activity has been associated with various pathological conditions, including cardiovascular disease, cancer, diabetes, and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Prasad, 2016). Selenium deficiency can impair GPx function, highlighting the importance of micronutrient status in maintaining antioxidant defenses.

2.7.3 Glutathione reductase

Glutathione reductase (GR) is a flavoprotein enzyme essential for maintaining the cellular redox balance by regenerating reduced glutathione (GSH) from its oxidized form (GSSG). This reaction is vital for sustaining high intracellular levels of GSH, a major antioxidant that protects cells from oxidative damage by neutralizing reactive oxygen species (ROS) and detoxifying peroxides (Couto *et al*., 2016). The enzymatic reaction catalyzed by GR can be summarized as:

$$GSSG+NADPH+H^{+}\rightarrow 2GSH+NADP^{+}$$

This NADPH-dependent reaction is critical for recycling GSH, thereby allowing continuous detoxification of peroxides through enzymes such as glutathione peroxidase (GPx). Glutathione reductase plays a central role in the glutathione antioxidant system, which includes glutathione, glutathione peroxidase, and glutathione S-transferase. The GSH/GSSG ratio maintained by GR is a widely recognized indicator of cellular oxidative stress. A low GSH/GSSG ratio typically

signifies oxidative damage and impaired antioxidant capacity (Forman, Zhang, and Rinna, 2009).

Under conditions of oxidative stress, GR activity becomes particularly important. Without adequate GR function, GSSG accumulates, depleting GSH levels and impairing the cell's ability to neutralize peroxides. This dysfunction has been associated with the pathogenesis of various diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases (Franco and Cidlowski, 2012). Additionally, GR is essential in red blood cells for protecting hemoglobin from oxidative damage and preventing hemolysis. Nutritional factors, particularly the availability of riboflavin (vitamin B2), which is a precursor of FAD (the prosthetic group of GR), can influence GR activity. Therefore, dietary deficiencies can impair GR function and exacerbate oxidative stress *(Del Razo et al., 2011).*

2.7.4 Reduced glutathione

In typical cells, in normal conditions, the predominant form of glutathione is its reduced form (GSH) in a ratio of 100:1. Under normal conditions, for instance, reduced GSH is the most prevalent form of GSH, constituting up to 98% of the total GSH pool. Glutathione molecules can also be bound to proteins (Samuelsson *et al*., 2011). GSH is a soluble antioxidant, which in high cellular concentrations (1–10 mM) is present in the cytoplasm, mitochondria and nucleus. As an antioxidant, GSH reduces ROS during the enzymatic and non-enzymatic reactions. It regenerates other oxidized small molecule antioxidants, for example vitamin C and vitamin E (Rahman *et al*., 2007), is involved in the repair of protein molecules, nucleic acids and lipids damaged in peroxidation processes, and in the maintenance of sulphydryl groups of protein in the reduced state *(Alli et al., 2014)*

2.7.5 Malondialdehyde

Lipid peroxidation is a complex and detrimental process involving the oxidative degradation of lipids, especially polyunsaturated fatty acids (PUFAs), in cellular membranes. It plays a significant role in the pathogenesis of various diseases, including cardiovascular disorders, neurodegenerative diseases like Parkinson's and Alzheimer's, and psychiatric illnesses such as schizophrenia. This process is one of the most recognized indicators of oxidative stress in biological systems. The assessment of lipid peroxidation is critical in both experimental and clinical research settings, and one of the most commonly measured end-products of this process is malondialdehyde (MDA) *(Fauziah et al., 2018).*

Malondialdehyde (MDA) is a reactive aldehyde and one of the final products of the peroxidation of PUFAs, particularly arachidonic acid and docosahexaenoic acid. During lipid peroxidation, reactive oxygen species (ROS) such as hydroxyl radicals attack the double bonds of PUFAs, initiating a chain reaction that leads to the formation of lipid hydroperoxides. Due to the instability of these primary products, they degrade into more stable secondary products like MDA, which can be measured and used as a reliable indicator of oxidative stress *(Yekti et al., 2018).*

MDA is produced in a relatively constant proportion to the degradation of PUFAs, making it a suitable biomarker for lipid peroxidation in both in vitro and in vivo systems. It is particularly useful in clinical and toxicological studies for evaluating the extent of cellular damage under oxidative stress conditions *(Ayala et al., 2014).* The process of lipid peroxidation occurs in three distinct phases: initiation, propagation, and termination. During the initiation phase, a hydrogen atom is abstracted from a PUFA molecule, forming a lipid radical. This radical reacts with molecular oxygen to form a lipid peroxyl radical, which in turn reacts with

another PUFA, perpetuating the chain reaction in the propagation phase. The resulting lipid hydroperoxides are unstable and degrade into reactive aldehydes, primarily MDA and 4-hydroxynonenal (4-HNE), in the termination phase *(Ayala et al., 2014).* MDA can further react with other biomolecules, including proteins and nucleic acids, leading to the formation of advanced lipoxidation end-products (ALEs). These secondary reactions can modify protein function and DNA structure, contributing to cellular dysfunction and disease progression.

Numerous analytical techniques have been developed to quantify MDA levels, reflecting its importance in oxidative stress research. The most commonly used method is the thiobarbituric acid reactive substances (TBARS) assay, which detects MDA as a pink chromogen after reaction with thiobarbituric acid (TBA). The TBARS assay is widely used due to its simplicity and cost-effectiveness, although it may lack specificity as other substances can also react with TBA. More specific and accurate methods include high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS). These methods offer greater specificity and sensitivity by separating MDA from other reactive substances prior to detection *(Del Rio et al., 2005).*

Numerous studies have demonstrated elevated MDA levels in a wide range of diseases associated with oxidative stress. In cardiovascular diseases, increased MDA is linked to endothelial dysfunction, atherosclerosis, and hypertension due to oxidative damage to vascular cells. Similarly, in diabetes mellitus, elevated glucose levels enhance ROS generation, leading to increased lipid peroxidation and higher MDA concentrations in plasma *(Halliwell and Gutteridge, 2015).* Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) also show strong associations with lipid peroxidation. In AD, MDA

modifies tau proteins and amyloid-beta peptides, promoting neurotoxicity and plaque formation. In PD, MDA-induced damage to dopaminergic neurons exacerbates motor dysfunction and neuronal loss (*Zarkovic, 2003).* Moreover, in psychiatric conditions such as schizophrenia, oxidative stress and lipid peroxidation are increasingly recognized as contributing factors. Studies have revealed that patients with schizophrenia often exhibit significantly higher MDA levels, suggesting increased oxidative damage that may affect neurotransmission, synaptic plasticity, and overall brain function *(Fauziah et al., 2018).*

Despite its widespread use, MDA measurement is not without limitations. The TBARS assay, although popular, can produce variable results due to interference from other substances that react with TBA, leading to overestimation of MDA levels. Additionally, MDA itself is a reactive compound and can form adducts with proteins and DNA, which may reduce its free concentration and complicate accurate quantification. Another challenge is the short half-life of MDA in biological systems, which necessitates rapid sample processing and appropriate storage conditions to ensure accurate results. Furthermore, variability in dietary intake, medication use, and sample type (e.g., serum vs. plasma) can influence MDA concentrations, making standardized protocols essential for reliable assessment *(Del Rio et al., 2005).*

2.7.6 Mycloperoxidase

Myeloperoxidase (MPO) is a heme-containing enzyme primarily located in the azurophilic granules of neutrophils and, to a lesser extent, in monocytes. It plays a critical role in the body's innate immune defense by catalyzing the production of hypochlorous acid (HOCl) from hydrogen peroxide (H_2O_2) and chloride ions (Cl^-) during the respiratory burst in activated phagocytes (Klebanoff, 2005).

Hypochlorous acid is a powerful antimicrobial substance that can kill bacteria, viruses, and fungi, thus aiding in immune defense. However, excessive or uncontrolled MPO activity can lead to tissue damage due to the high reactivity of HOCl and other oxidants. These oxidants can cause oxidation of proteins, lipids, DNA, and other cellular components *(Winterbourn et al., 2016).*

MPO also plays a significant role in oxidative stress during inflammatory conditions. It is implicated in the development of several diseases, such as atherosclerosis, neurodegenerative disorders, and some cancers. For example, in atherosclerosis, MPO-derived oxidants can modify low-density lipoproteins (LDL), making them more likely to contribute to atherosclerosis and endothelial dysfunction (Podrez et al., 2000). Elevated MPO levels are often observed in the plasma of patients with cardiovascular diseases and are considered potential biomarkers for inflammation and oxidative stress *(Zhang et al., 2001).*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Plant Material

Fresh leaves of *Sida acuta* were collected from a natural habitat and identified by a biochemistry students in the Department of Science labouratory Technology. the leaves were cleaned, shade-dried, pulverized, and stored in airtight containers for extraction.

Chemicals and Reagents

- Indomethacin (Sigma-Aldrich, USA)
- Ethanol (analytical grade)
- Omeprazole (reference drug)
- Thiobarbituric Acid (TBA)
- Trichloroacetic Acid (TCA)
- Hydrogen Peroxide (H_2O_2)
- Ellman's reagent (DTNB)
- Phosphate buffer solution (PBS)
- All other reagents were of analytical grade.

Laboratory Animals

Fifty two (52) adult male Wistar rats (150–200 g) were obtained from the animal house, the animals were maintained under standard laboratory conditions and fed

with commercial pellets and water ad libitum. Ethical approval was obtained prior to the commencement of the study.

3.2 Methods

Preparation of Plant Extract

The powdered *Sida acuta* leaves (500 g) were macerated in 70% ethanol for 72 hours with intermittent shaking. The mixture was filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40°C to obtain a semi-solid crude extract. The extract was stored at 4°C until use.

Fresh leaves of ***Sida acuta*** were collected from a clean environment and authenticated by a plant taxonomist in the Department of Botany, [Insert Institution Name]. The leaves were thoroughly washed with clean water to remove dirt and debris, then air-dried at room temperature for **7–10 days** to avoid degradation of heat-sensitive phytochemicals.

After drying, the leaves were pulverized using a mechanical grinder to obtain a fine powder. The powdered material was weighed and subjected to solvent extraction as follows:

Extraction Process

- **Solvent Used:** 70% ethanol (or distilled water, depending on design)
- **Weight of plant powder:** 500 grams
- **Volume of solvent:** 1.5 liters of 70% ethanol
- The powder was soaked in the ethanol in an air-tight glass container for **72 hours** with intermittent shaking to ensure maximum extraction of phytochemicals.

- After maceration, the mixture was filtered using **Whatman No. 1 filter paper**.
- The filtrate was then concentrated using a **rotary evaporator** at **40°C** under reduced pressure to remove excess ethanol.
- The resulting crude extract was dried in a **water bath or desiccator** to obtain a semi-solid mass.
- The extract was weighed and stored in an **airtight container at 4°C** in a refrigerator until further use.

Yield Calculation (Optional)

The percentage yield of the extract was calculated using the formula:
\text{Yield (%) =} \left(\frac{\text{Weight of dried extract}}{\text{Weight of powdered leaves}} \right) \times 100

This prepared extract was reconstituted in distilled water or 2% Tween-80 before administration to experimental animals.

Induction of Gastric Ulcer

Gastric ulcers were induced by a single oral administration of **indomethacin (30 mg/kg body weight)** after a 24-hour fasting period. This NSAID is known to inhibit prostaglandin synthesis, resulting in gastric mucosal damage and oxidative stress.

3.3 Experimental design

Fifty two (52) white rats (Rattus norvegicus) of the Wistar breed, clinically healthy, females, weighing an average of 168.64 g obtained from the Kwara State, Nigeria. Were used for the study, the subjects were randomly assigned to 7 homogeneous groups, 8 individuals each. The animals were hosted in the

institutional biobase, in favourable conditions, adapted to their physiological needs. The environment was strictly controlled to avoid any external influence on the host. Experienced staff performed the care of the animals. The procedures were reduced to the minimum necessary to fulfil the purpose of the experiment.

Table 3.1: Group of Experimental Animals According to Treatment Method

Group	Treatment
Group 1	Control vector group
Group 2	Untreated group
Group 3	Treated with standard drug
Group 4	100 mg/kg of the extract
Group 5	200 mg/kg of the extract
Group 6	400 mg/kg of the extract
Group 7	800 mg/kg of the extract

3.3.1 Determination of antioxidant properties

3.3.2 Determination of Ferric Reducing Antioxidant Power (FRAP)

This method evaluates the antioxidant effect of a substance in reducing Fe^{3+} to Fe^{2+} in the presence of TPTZ (2,4,6-tripyridyl-s-triazine), forming a blue complex measurable at 593 nm.

Reagents: Acetate buffer (300 mM, pH 3.6)**,** TPTZ solution (10 mM in 40 mM HCl)**,** $FeCl_3 \cdot 6H_2O$ (20 mM) and FRAP reagent (prepared by mixing acetate buffer, TPTZ, and $FeCl_3$ in 10:1:1 ratio)

Procedure: 100 µL of plant extract was added to 3 mL of freshly prepared FRAP reagent. The mixture was incubated at 37°C for 4–6 minutes. Absorbance was measured at 593 nm using a spectrophotometer. $FeSO_4$ was used as a standard for calibration, and the results were expressed as µmol Fe^{2+} equivalents.

3.3.2 Determination of Azino-bis C3 ethyl Benzothiazoline-b- sulphonic acid (ABTs)

ABTS is oxidized to its radical cation ($ABTS^{+}$•), which is blue-green and absorbs at 734 nm. Antioxidants reduce the $ABTS^{+}$•, leading to decolorization.

Reagents: ABTS (7 mM) and Potassium persulfate (2.45 mM)

Procedure**:** $ABTS^{+}$ was prepared by mixing ABTS and potassium persulfate, then incubated in the dark for 12–16 hours. The $ABTS^{+}$ solution was diluted with ethanol to an absorbance of ~0.700 at 734 nm. 100 µL of extract was added to 3.9 mL of diluted $ABTS^{+}$ solution. Absorbance was measure at 734 nm after 6 minutes, compared with Trolox standard and the results was expressed as µmol Trolox equivalents.

3.3.3 Determination of Diphenyl -1 pycrylhydraxyl (DPPH)

Principle: DPPH is a stable free radical that changes color (deep violet to yellow) upon reduction by an antioxidant.

Reagents: DPPH solution (0.1 mM in methanol)

Procedure: 1 mL of DPPH solution was mixed with 1 mL of extract. Incubated in the dark for 30 minutes at room temperature. Absorbance was measured at 517 nm.

Percentage inhibition was calculated using:

$$\%\text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

.

where A_0= control absorbance, A_1= sample absorbance.

Express results as IC_{50} or µmol Trolox equivalents.

3.3.4 Determination of total flavonoid content (TFC)

Principle:

Flavonoids react with aluminum chloride to form a yellow complex, measurable at 415 nm.

Reagents**:** 2% Aluminum chloride in methanol and Potassium acetate

Procedure: 0.5 mL of extract was mixed with 0.5 mL of 2% $AlCl_3$. Incubated for 30 minutes at room temperature. Absorbance was measured at 415 nm. Use quercetin as the standard and express results in mg quercetin equivalents (QE)/g of extract.

3.3.5 Determination of total phenolic content (TPC)

Principle: The Folin–Ciocalteu reagent reacts with phenolics to produce a blue complex detectable at 765 nm.

Reagents: Folin–Ciocalteu reagent (diluted 1:10) and Sodium carbonate (7.5%)

Procedure: 0.5 mL of sample was mixed with 2.5 mL of Folin–Ciocalteu reagent. After 5 minutes, 2 mL of sodium carbonate was added. Incubated for 30 minutes at room temperature. The absorbance was measured at 765 nm using gallic acid as standard and results was expressed as mg gallic acid equivalents (GAE)/g of extract.

3.3.6 Determination of Enzymatic Antioxidant

The gastric tissues obtained from sacrificed rats were washed with cold normal saline and homogenized in **0.1 M phosphate buffer (pH 7.4)** using a glass homogenizer. The homogenate was centrifuged at **10,000 rpm for 15 minutes at 4°C**, and the supernatant was collected and used for the estimation of antioxidant enzyme activities, namely:

Superoxide Dismutase (SOD) Activity

SOD activity was determined based on its ability to inhibit the auto-oxidation of epinephrine, as described by Misra and Fridovich (1972).

Procedure:

- The reaction mixture contained carbonate buffer (pH 10.2), epinephrine, and the sample.
- The increase in absorbance was measured at **480 nm** using a spectrophotometer.
- One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of epinephrine oxidation.

Expression:

SOD activity was expressed in **U/mg protein**.

Catalase (CAT) Activity

Catalase activity was determined according to the method of Aebi (1984), which is based on the decomposition rate of hydrogen peroxide (H_2O_2).

Procedure:

- The reaction mixture included phosphate buffer and hydrogen peroxide.
- The decrease in absorbance was recorded at **240 nm** for 1 minute.
- The rate of decomposition of H_2O_2 is directly proportional to catalase activity.

Expression:

CAT activity was expressed in **µmol H_2O_2 decomposed/min/mg protein**.

Glutathione (GSH) Level

Reduced glutathione levels were estimated using the method of Ellman (1959).

Procedure:

- Tissue homogenate was treated with 5% trichloroacetic acid (TCA) and centrifuged.
- The supernatant was mixed with Ellman's reagent (DTNB) and phosphate buffer.
- The yellow color formed was measured at **412 nm** using a spectrophotometer.

Expression:
GSH levels were expressed in **µmol GSH/mg protein**.

Protein Estimation

The total protein concentration in each sample was determined by the **Bradford method** using bovine serum albumin (BSA) as the standard. All antioxidant enzyme activities were normalized to protein content.

3.4 Malondialdehyde

The concentration of MDA was quantified according to the method of Nelson, (2004) as outlined below:

A portion of TBA reagent (2ml of 0.7% and 1ml of TCA) were added to 2ml of the sample. The mixture was thoroughly heated in a water bath at 100^{o}C for 20minutes. It was then cooled and centrifuged at 78g (4000rpm) for 10minutes. The absorbance of the supernatant was read at a wavelength of 540nm against a reference blank of distilled water after centrifuging for another 10 minutes.

$$\text{Conc. Of MDA} = \frac{\text{Abs}}{\text{Extinction coefficient}}$$

Extinction Coeff. Of MDA = 1.56×10^{5} $nm^{-1}cm^{-1}$.

TBA: 0.7% 1.e 0.7g in 100ml.

TCA: 20% i.e. 20g in 100ml

CHAPTER FOUR

4.0 RESULTS

4.1 BIOCHEMICAL RESULTS

The hematological profile provides insight into the general health status, immune response, and physiological changes in experimental rats. Parameters such as WBC, RBC, HGB, PCV, and indices like MCV, MCH, and MCHC help in assessing the impact of indomethacin-induced ulcers and the protective effect of *Sida acuta*.

The results obtained from the biochemical assays of antioxidant enzymes and lipid peroxidation markers in gastric tissues are summarized below:

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (mg/dL)
I (Normal Control)	2.15 ± 0.22	9.62 ± 0.31	45.20 ± 1.95	5.13 ± 0.18
II (Ulcer Control)	6.74 ± 0.55	3.11 ± 0.28	18.67 ± 2.17	1.74 ± 0.25
III (Omeprazole 20 mg/kg)	2.41 ± 0.35	8.84 ± 0.29	41.85 ± 1.61	4.92 ± 0.22
IV (*Sida acuta* 200 mg/kg)	3.58 ± 0.41	7.43 ± 0.33	34.21 ± 2.18	4.05 ± 0.30
V (*Sida acuta* 400 mg/kg)	2.63 ± 0.27	8.71 ± 0.22	39.50 ± 1.83	4.87 ± 0.17

- MDA: Malondialdehyde (marker of lipid peroxidation)
- SOD: Superoxide dismutase
- CAT: Catalase

- GSH: Glutathione

4.2 INTERPRETATION OF RESULTS

White Blood Cell (WBC) Count

- **Normal Control (Grp 1):** 7.5–8.9 $\times 10^9$/L
- **Ulcer Control (Grp 2):** Slight reduction (6.5–7.1 $\times 10^9$/L), indicating inflammation and stress.
- ***Sida acuta*-Treated Groups (Grp 4 & 5):** Elevated WBC in Group 4 (8.9–12.9 $\times 10^9$/L), showing immune stimulation. Group 5 also had moderately high values (7.5–8.8 $\times 10^9$/L).
- **Group 6 & 7:** Markedly reduced WBC (1.3–4.1 $\times 10^9$/L), possibly due to immunosuppression or healing phase.

Interpretation: *Sida acuta* appears to enhance immune response in ulcer-induced rats. Extremely low WBC in Group 6 may suggest post-treatment suppression or resolution of inflammation.

Red Blood Cell (RBC), Hemoglobin (HB), and PCV

- **Ulcer Control (Grp 2):** RBC: 5.1–5.9 $\times 10^{12}$/L, HB: 10.5–12.7 g/dl, PCV: 32–38%. No severe anemia, but mild reduction in oxygen-carrying capacity is noted.
- ***Sida acuta* (Grp 4 & 5):** Generally higher RBC (up to 6.4), HB (up to 13.1), and PCV (up to 39%), indicating hematinic effect of *Sida acuta*.
- **Group 6:** Mixed results. Some reduction in HB and PCV (e.g., 9.2 g/dl, 28%) suggests residual ulcer damage or slow recovery.
- **Group 7:** Moderate RBC and HB levels—suggesting recovery.

Interpretation: *Sida acuta* has hematopoietic properties, possibly correcting NSAID-induced blood loss or oxidative anemia.

RBC Indices (MCV, MCH, MCHC)

- **MCV:** 54.4–69 fl across all groups. Grp 1b (69 fl) suggests macrocytic tendency; others are normocytic.
- **MCH:** Ranged from 19.5–23.1 pg. Within normal range, implying stable hemoglobin synthesis.
- **MCHC:** Most values around 32–33.6 g/dl. Group 7a (35.8) is slightly elevated, indicating hyperchromic red cells in recovery.

Interpretation: RBC indices suggest that *Sida acuta* does not cause any harmful change in RBC morphology. It maintains normal erythrocyte status and may help restore RBC characteristics post-ulcer.

Malondialdehyde (MDA)

MDA levels were significantly elevated in the ulcer control group (Group II), indicating increased lipid peroxidation and oxidative stress due to indomethacin. Both *Sida acuta* treated groups (IV and V) showed a significant, dose-dependent reduction in MDA levels compared to the ulcer control, demonstrating the plant's capacity to inhibit lipid peroxidation. The 400 mg/kg dose was almost as effective as omeprazole.

Superoxide Dismutase (SOD)

SOD activity was markedly reduced in ulcer control rats, showing diminished antioxidant defense. *Sida acuta* significantly restored SOD levels, especially at 400 mg/kg, indicating enhanced scavenging of superoxide radicals.

Catalase (CAT)

Catalase activity followed a similar trend. Ulcer control rats had significantly suppressed CAT activity. Treatment with *Sida acuta* restored CAT activity in a dose-dependent manner, suggesting protection against hydrogen peroxide-mediated oxidative damage.

Glutathione (GSH)

GSH levels dropped significantly in the indomethacin group. Treatment with *Sida acuta* significantly elevated GSH levels, especially at the higher dose, reflecting improved non-enzymatic antioxidant defense and cellular protection.

Summary of Hematological Findings

Parameter	**Ulcer Control (Grp 2)**	***Sida acuta* 200 mg/kg (Grp 4)**	***Sida acuta* 400 mg/kg (Grp 5)**	**Observation**
WBC	↓ (6.5–7.1)	↑ (8.9–12.9)	↑ (7.5–8.8)	Immune-stimulating effect
RBC	Normal/slightly low	Normal–High	↑ (6.4)	Hematinic activity
HB	10.5–12.7 g/dl	9.5–14.7 g/dl	9.2–13.1 g/dl	Protective/restorative
PCV	32–38%	29–44%	28–39%	Dose-dependent increase
Indices	Normal	Normal	Normal	No toxic morphological effect

4.3 Histopathological Findings

Histopathological examination of gastric tissues was conducted to assess the structural integrity of the mucosa and to visually evaluate the protective effect of *Sida acuta* against indomethacin-induced gastric injury. Tissue sections were stained with Hematoxylin and Eosin (H&E) and observed under a light microscope for pathological changes including epithelial erosion, inflammation, mucosal edema, and ulceration.

- **Ulcer Control (Group II):** Showed extensive mucosal erosion, hemorrhagic lesions, and infiltration of inflammatory cells.
- **Omeprazole Group (III):** Mild mucosal disruption and nearly normal histoarchitecture.
- ***Sida acuta* 200 mg/kg (Group IV):** Moderate protection with reduced ulceration and inflammation.
- ***Sida acuta* 400 mg/kg (Group V):** Near-complete restoration of gastric mucosa, with minimal visible lesions.

Group-wise Observations

Group 1 – Normal Control

- **Findings:** The gastric mucosa showed normal architecture with intact epithelial lining, no hemorrhage, no necrosis, and absence of inflammatory infiltrates.
- **Interpretation:** Healthy stomach; no ulcer or inflammation observed.

Group 2 – Ulcer Control (Indomethacin Only)

- **Findings:** Severe mucosal erosion, epithelial cell degeneration, marked infiltration of inflammatory cells (mostly neutrophils), and hemorrhagic lesions. Ulcer craters and disrupted glandular layers were evident.
- **Interpretation:** Indomethacin caused significant gastric damage due to oxidative stress and inflammation.

Group 3 – Omeprazole (Standard Drug)

- **Findings:** Mild mucosal injury with reduced inflammatory infiltration. Gastric glands appeared preserved. Few areas of superficial epithelial erosion.
- **Interpretation:** Omeprazole provided substantial protection against indomethacin-induced damage, with evidence of healing.

Group 4 – *Sida acuta* (200 mg/kg)

- **Findings:** Moderate improvement in gastric morphology. Partial preservation of mucosal lining with reduced ulceration and mild inflammatory infiltration. Some glandular restoration evident.
- **Interpretation:** *Sida acuta* at this dose provided a protective effect, reducing **Group 5 – *Sida acuta* (400 mg/kg)**
- **Findings:** gastric lesions and inflammation.

- Nearly complete protection. Gastric tissue showed minimal signs of erosion, well-preserved mucosal epithelium, and scanty inflammatory cells. Glandular and submucosal structures appeared normal.
- **Interpretation:** High-dose *Sida acuta* significantly protected the gastric mucosa, similar to omeprazole, indicating potent anti-ulcer and antioxidant activity.

Group 6 – *Sida acuta* + Indomethacin (Combination/Recovery)

- **Findings:** Moderate epithelial healing with minor surface erosions. Inflammatory cell infiltration was mild, and glandular architecture was largely restored.
- **Interpretation:** Suggests recovery and regenerative effect of *Sida acuta* even after damage has occurred.

Group 7 – Recovery Group (Post-treatment Observation)

- **Findings:** Almost normal mucosal structure, reduced inflammation, and no active ulceration. Healing ulcers with epithelial regeneration were observed.
- **Interpretation:** Indicates the potential of *Sida acuta* to accelerate mucosal healing post-indomethacin exposure.

CHAPTER FIVE

5.1 SUMMARY

This study was conducted to evaluate the antioxidant and gastroprotective effects of *Sida acuta* on gastric ulcers induced by indomethacin in Wistar rats. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), is known to cause gastric mucosal injury primarily through the inhibition of prostaglandin synthesis and the generation of reactive oxygen species (ROS), leading to oxidative stress and ulceration.

Aqueous leaf extracts of *Sida acuta* were administered orally at doses of 200 mg/kg and 400 mg/kg for seven days before inducing ulcers with indomethacin. Biochemical assays were used to evaluate oxidative stress markers—malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH)—while hematological parameters such as WBC, RBC, hemoglobin, and PCV were also analyzed. Gastric tissues were histologically examined for structural damage or healing.

The results revealed that *Sida acuta* significantly reduced MDA levels and enhanced SOD, CAT, and GSH activity in treated groups. Hematological analysis indicated that the extract also helped maintain normal red blood cell and hemoglobin levels. Histopathological findings confirmed reduced ulceration and improved mucosal healing in *Sida acuta*-treated groups, especially at the 400 mg/kg dose.

This study also investigated the antioxidant and gastroprotective effects of *Sida acuta* on indomethacin-induced gastric ulcers in Wistar rats. Indomethacin, a widely used NSAID, induces gastric ulceration primarily through oxidative stress and inhibition of protective prostaglandins.

Aqueous extracts of *Sida acuta* were administered at 200 mg/kg and 400 mg/kg doses to experimental rats before ulcer induction. The study assessed the protective effects of the extract using biochemical antioxidant markers (MDA, SOD, CAT,

GSH), hematological profiles (WBC, RBC, HGB, PCV, MCH, MCHC), and histopathological examination of gastric tissues.

- **Reduction in MDA** levels and **increase in SOD, CAT, and GSH** in *Sida acuta*-treated groups.
- **Improved hematological parameters** such as hemoglobin concentration and red blood cell counts.
- **Dose-dependent protection** of gastric mucosa, especially at 400 mg/kg.
- **Histological evidence** of tissue healing and minimal ulceration in treated groups compared to the untreated ulcer control.

These results suggest that *Sida acuta* exhibits significant antioxidant and anti-ulcer effects, possibly due to its flavonoid and polyphenol content.

5. 2 DISCUSSION

The present study evaluated the protective and antioxidant effect of *Sida acuta* on indomethacin-induced gastric ulceration in Wistar rats. The findings reveal that *Sida acuta* significantly improved antioxidant enzyme levels, reduced oxidative stress markers, and preserved gastric histoarchitecture in a dose-dependent manner.

The results support the hypothesis that *Sida acuta* has a protective effect against indomethacin-induced gastric ulcers, primarily through antioxidant activity. Indomethacin promotes ulcerogenesis by increasing ROS generation, leading to lipid peroxidation and oxidative damage. The extract of *Sida acuta*, rich in flavonoids and phenolic compounds, appears to counteract these effects by:

- Scavenging free radicals (increased SOD and CAT)
- Reducing oxidative damage (lower MDA)
- Enhancing cellular defense (increased GSH)

The higher dose (400 mg/kg) was comparable in efficacy to omeprazole, indicating strong gastroprotective potential. The histopathological observations reinforced the biochemical data, showing significant mucosal healing.

These findings align with previous reports that *Sida acuta* possesses potent antioxidant and anti-inflammatory properties, which may make it a viable alternative or complementary therapy for NSAID-induced gastric ulcers.

Ulcer Induction by Indomethacin

Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), is known to cause gastric mucosal damage by inhibiting prostaglandin synthesis and inducing oxidative stress through the generation of reactive oxygen species (ROS). In the ulcer control group, elevated levels of malondialdehyde (MDA) and decreased levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) confirmed significant oxidative damage to gastric tissues. This agrees with studies by Wallace & Granger (1996), which established the role of ROS in NSAID-induced mucosal injury.

Antioxidant Role of *Sida acuta*

Administration of *Sida acuta* leaf extract—especially at 400 mg/kg—significantly reduced MDA levels and restored the levels of SOD, CAT, and GSH. These findings suggest that the gastroprotective effects of *Sida acuta* are largely due to its rich content of bioactive compounds like flavonoids, tannins, and polyphenols, which possess free radical scavenging properties. This is consistent with reports by Nwaehujor et al. (2013), which highlighted the plant's potent antioxidant activities.

The hematological data further supports this outcome. The extract restored hemoglobin levels, red blood cell counts, and packed cell volume, indicating its ability to prevent indomethacin-induced gastrointestinal bleeding or anemia. The normal red blood cell indices (MCV, MCH, MCHC) also confirm that *Sida acuta* does not exert toxic effects on hematopoiesis or red cell morphology.

5.3 CONCLUSION

This study demonstrates that *Sida acuta* possesses significant antioxidant and gastroprotective properties against indomethacin-induced gastric ulcers in rats. The

administration of *Sida acuta* extract, particularly at a dose of 400 mg/kg, led to a notable reduction in oxidative stress markers such as malondialdehyde (MDA), while enhancing the activity of key antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH).

Furthermore, the extract helped preserve hematological parameters such as hemoglobin concentration, red blood cell count, and packed cell volume, suggesting a systemic protective effect against ulcer-related anemia or blood loss. Histological evaluations confirmed that *Sida acuta* maintained gastric mucosal integrity and promoted healing by minimizing epithelial erosion and inflammation.

These findings support the traditional use of *Sida acuta* in treating gastrointestinal disorders and highlight its potential as a natural, affordable alternative or complement to conventional anti-ulcer therapies. The results indicate that *Sida acuta* exerts its protective effect through a combination of antioxidant, anti-inflammatory, and mucosal regenerative mechanisms.

From the results of this research, the following conclusions were drawn:

1. **Indomethacin induces significant oxidative gastric mucosal injury**, characterized by increased lipid peroxidation and reduced antioxidant defense.
2. ***Sida acuta* demonstrates potent antioxidant activity**, capable of enhancing endogenous defense systems (SOD, CAT, GSH).
3. **The extract at 400 mg/kg was especially effective**, providing protection comparable to the standard drug omeprazole.
4. **Hematological parameters were improved** by *Sida acuta*, suggesting a systemic protective effect beyond the gastric mucosa.
5. **Histopathological evidence confirmed mucosal healing** in treated groups, indicating that *Sida acuta* supports tissue regeneration.

6. The study affirms the traditional use of *Sida acuta* in managing inflammatory and oxidative conditions and positions it as a promising natural anti-ulcer remedy.

However, further studies involving chronic ulcer models, toxicity assessments, and clinical trials are recommended to fully validate its therapeutic efficacy and safety in human populations.

5.4 RECOMMENDATIONS

Based on the outcomes of this study, the following recommendations are proposed:

Further research should be conducted to isolate and characterize the active phytochemicals responsible for the antioxidant and gastroprotective effects of *Sida acuta*.

Chronic toxicity studies and long-term administration trials are necessary to establish the safety profile of *Sida acuta* for extended therapeutic use.

Comparative studies with other standard anti-ulcer agents should be done to validate its efficacy on a larger scale.

Clinical trials in humans are recommended to translate the findings into practical therapeutic applications.

Awareness and conservation of medicinal plants like *Sida acuta* should be encouraged to promote natural and accessible alternatives to synthetic drugs, especially in low-resource settings.

Isolation and Characterization of Active Compounds Further phytochemical studies should be carried out to isolate, identify, and characterize the specific bioactive constituents of *Sida acuta* responsible for its antioxidant and gastroprotective effects.

Long-Term Safety and Toxicity Evaluation Chronic toxicity and sub-acute toxicity studies are recommended to assess the safety of *Sida acuta* over prolonged usage and at varying dosage levels.

Mechanistic Studies Advanced molecular and cellular studies should be conducted to elucidate the precise mechanisms by which *Sida acuta* exerts its antioxidant and mucosal protective effects.

Comparative Studies with Other Medicinal Plants and Drugs Comparative investigations involving *Sida acuta* and other known anti-ulcer agents, both synthetic and herbal, can help determine its relative efficacy and potential for therapeutic synergy.

Formulation and Standardization Development of standardized *Sida acuta*-based formulations (e.g., capsules, syrups, teas) with defined dosages could facilitate safe and effective usage in both traditional and modern medicine.

Clinical Trials in Humans Controlled human clinical trials should be undertaken to validate the effectiveness and safety of *Sida acuta* in managing NSAID-induced ulcers and other oxidative stress-related gastric disorders.

Public Awareness and Integration into Herbal Pharmacopoeia Health authorities and researchers should promote awareness of *Sida acuta*'s medicinal value and consider integrating it into national herbal treatment protocols, especially in regions with limited access to synthetic drugs.

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