EFFECTS OF *Sida acuta* LEAF EXTRACT ON LIVER FUNCTION AND INDOMETHACIN-INDUCED ULCEROGENIC RATS

BY

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BEING A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, (BIOCHEMISTRY UNIT), INSTITUTE OF APPLIED SCIENCES, KWARA STATE POLYTECHNIC, ILORIN

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CERTIFICATION

This is to certify that this research project work was carried out by **OLADIPUPO BARAKAT OPEYEMI** with matric. Number **HND/23/SLT/FT/0853**, submitted to Department of Science Laboratory Technology, Institute of Applied Sciences (IAS), Kwara State Polytechnic, Ilorin, in partial fulfillment for the requirement of award of Higher National Diploma (HND) in Science Laboratory Technology.

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DEDICATION

This project is dedicated to the Almighty God, whose grace, strength, and wisdom guided me through every stage of this research.

I also dedicate this work to my loving parents and family, whose constant prayers, support, and encouragement have been my backbone throughout this academic journey.

Finally, I dedicate this project to all aspiring scientists and researchers who are committed to the pursuit of knowledge, especially in the field of biomedical research.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Gastric ulcers, a significant global health concern, arise from an imbalance between aggressive factors such as gastric acid, pepsin, and reactive oxygen species (ROS) and protective mechanisms like mucus, bicarbonate, and prostaglandins in the gastric mucosa. Nonsteroidal anti-inflammatory drugs (NSAIDs), particularly indomethacin, are a primary cause of gastric ulcers due to their inhibition of cyclooxygenase (COX) enzymes, which reduces prostaglandin synthesis and increases oxidative stress, leading to mucosal damage (Zaghlool *et al*., 2015). These ulcers not only cause pain and discomfort but also pose risks of severe complications, including bleeding and perforation, contributing to approximately 15,000 deaths annually worldwide (Malfertheiner *et al*., 2009). Additionally, NSAID-induced systemic inflammation and oxidative stress can impair liver function, elevating enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are critical indicators of hepatic health (Akindele *et al*., 2022).

Conventional treatments, including proton pump inhibitors (PPIs) like omeprazole and H2-receptor antagonists, effectively manage gastric ulcers by reducing acid secretion but are associated with side effects such as hepatotoxicity, nutrient malabsorption, and high costs, particularly in resource-limited settings like Nigeria (Bi *et al*., 2014). This has driven interest in herbal remedies, which offer potential safety and affordability. *Sida acuta* (Malvaceae), a medicinal plant widely used in traditional African and Asian medicine, is known for its antioxidant, anti-inflammatory, and mucoprotective properties, attributed to bioactive compounds like flavonoids, alkaloids, and tannins (Oboh & Onwukaeme, 2005). While *Sida acuta* has shown hepatoprotective effects in models like carbon tetrachloride (CCl4)-induced liver damage, its specific impact on indomethacin-induced gastric ulcers and associated liver dysfunction remains underexplored. This study investigates the anti-ulcerogenic and hepatoprotective effects of *Sida acuta* leaf extract in

Wistar rats, aiming to provide empirical evidence for its therapeutic potential and challenge the pharmaceutical-centric narrative in ulcer management.

Gastric ulcers are a prevalent gastrointestinal disorder, affecting millions globally, with a higher burden in developing countries due to widespread NSAID use and limited healthcare access. Indomethacin, a potent NSAID, is commonly used in experimental models to induce gastric ulcers in rats, mimicking human NSAID-induced ulcerogenesis through COX inhibition, reduced prostaglandin levels, increased acid secretion, and elevated ROS, leading to mucosal erosion and oxidative stress (Sabiu *et al*., 2015). These ulcers disrupt the gastric mucosal barrier, causing pain, bleeding, and, in severe cases, perforation, with a global incidence of 0.1–0.3% annually (Malfertheiner *et al*., 2009). Beyond gastric damage, indomethacin's systemic effects can impair liver function by inducing oxidative stress and inflammation, elevating liver enzymes (ALT, AST, alkaline phosphatase [ALP]) and bilirubin, which reflect hepatic injury (Akindele *et al*., 2022).

Current treatments rely heavily on synthetic drugs like PPIs and H2-receptor antagonists, which reduce gastric acid secretion and promote ulcer healing. However, their long-term use is linked to adverse effects, including hepatotoxicity, renal dysfunction, and increased risk of infections, particularly in vulnerable populations (Bi *et al*., 2014). These limitations have prompted exploration of herbal alternatives, which are often safer and more accessible. *Sida acuta*, commonly known as wireweed, is a medicinal plant with a rich history in traditional medicine for treating digestive disorders, inflammation, and oxidative stress-related conditions. Its leaves contain flavonoids, tannins, and alkaloids, which exhibit antioxidant properties by scavenging ROS, reducing lipid peroxidation (measured as MDA), and enhancing enzymatic defenses like SOD, catalase (CAT), and glutathione (GSH) (Oboh & Onwukaeme, 2005). Previous studies have confirmed *Sida acuta*'s hepatoprotective effects in CCl4-induced liver damage models, where it significantly reduced liver enzyme levels and oxidative stress markers (Akindele *et al*., 2022). However, its potential in mitigating indomethacin-induced gastric ulcers and associated liver dysfunction remains largely unstudied, presenting a critical research gap.

The pharmaceutical industry's focus on synthetic drugs often marginalizes herbal remedies like *Sida acuta*, potentially due to limited research funding and a bias toward patentable compounds. This study seeks to bridge this gap by evaluating *Sida acuta*'s effects on indomethacin-induced ulcerogenic rats, with a focus on both gastric and hepatic outcomes, to provide a scientific basis for its integration into ulcer management strategies.

1.2 Statement of Research Problem

Indomethacin-induced gastric ulcers represent a significant health challenge, particularly in regions like Nigeria, where NSAID use is prevalent due to chronic pain conditions and limited access to alternative treatments. These ulcers cause mucosal damage, leading to complications such as bleeding and perforation, with a global mortality rate of approximately 15,000 annually (Malfertheiner *et al*., 2009). The systemic effects of indomethacin, including oxidative stress and inflammation, also impair liver function, elevating markers like ALT, AST, ALP, and bilirubin, which can exacerbate health risks in ulcer patients (Zaghlool *et al*., 2015). While synthetic drugs like omeprazole effectively manage ulcers, their side effects, including hepatotoxicity and high costs, limit their suitability, especially in low-resource settings (Bi *et al*., 2014).

Sida acuta, a traditional remedy with antioxidant and anti-inflammatory properties, has shown promise in hepatoprotective models, reducing liver damage and oxidative stress markers (Akindele *et al*., 2022). However, there is a lack of empirical data on its efficacy against indomethacin-induced gastric ulcers and its ability to mitigate associated liver dysfunction. This research gap hinders the validation of *Sida acuta* as a viable therapeutic option and its integration into clinical practice. The reliance on pharmaceutical treatments, coupled with insufficient research on herbal alternatives, reflects a potential bias in the medical establishment, necessitating studies to explore natural remedies. This study addresses these issues by investigating *Sida acuta*'s effects on gastric ulcers and liver function in an indomethacin-induced rat model.

1.3 Justification for the Study

The study is justified on several grounds:

- **Public Health Need**: NSAID-induced gastric ulcers are a significant burden in Nigeria, with high morbidity due to limited access to safe, affordable treatments. Validating *Sida acuta* as an alternative could improve outcomes for patients with limited resources.
- **Scientific Gap**: While *Sida acuta* has demonstrated hepatoprotective effects in other models, its role in indomethacin-induced ulcers and associated liver dysfunction is underexplored, warranting empirical investigation.
- **Economic Benefit**: As a locally abundant plant, *Sida acuta* offers a cost-effective alternative to expensive synthetic drugs, potentially reducing healthcare costs in developing countries.
- **Safety Considerations**: Unlike PPIs, which may cause hepatotoxicity and other side effects, *Sida acuta*'s natural compounds may offer a safer profile, supporting its evaluation as a therapeutic agent (Bi *et al*., 2014).
- **Critical Perspective**: The study challenges the pharmaceutical industry's dominance in ulcer treatment, questioning the underfunding of herbal research and advocating for evidence-based integration of traditional remedies.

By providing scientific evidence on *Sida acuta*'s efficacy, this study aims to promote its use in ulcer management and contribute to reducing the reliance on potentially harmful synthetic drugs.

1.4 Aim and Objectives of the Study

Aim

The aim of this study is to investigate the effect of *Sida acuta* leaf extract on liver function and indomethacin-induced gastric ulcers in Wistar rats, with a view to establishing its potential as a therapeutic agent.

Objectives

The specific objectives are:

1. To evaluate the anti-ulcerogenic effect of *Sida acuta* leaf extract on indomethacin-induced gastric ulcers in rats.
2. To assess the impact of *Sida acuta* leaf extract on liver function indices (ALT, AST, ALP, total bilirubin) in ulcerogenic rats.
3. To determine the antioxidant (SOD, CAT, GSH, MDA) and anti-inflammatory (e.g., TNF-α, IL-6) mechanisms underlying *Sida acuta*'s effects.
4. To compare the efficacy of *Sida acuta* leaf extract with omeprazole in healing gastric ulcers and protecting liver function.
5. To propose *Sida acuta* as a viable alternative for managing NSAID-induced ulcers and associated liver dysfunction.

CHAPTER TWO

2.0 LITERATURE REVIEW

This literature review explores the pathophysiology of ulcers, their various types, and their relevance to the study of *Sida acuta*'s effects on indomethacin-induced ulcerogenic rats and liver function. Ulcers, characterized by localized tissue damage, pose significant health challenges globally, with gastric ulcers being a primary focus due to their association with nonsteroidal anti-inflammatory drugs (NSAIDs) like indomethacin. The review synthesizes current knowledge on ulcers, emphasizing their epidemiology, mechanisms, and the need for safer therapeutic alternatives like *Sida acuta*, a medicinal plant with antioxidant and anti-inflammatory properties. By examining different ulcer types, this chapter establishes a foundation for understanding indomethacin-induced gastric ulcers and their systemic effects, including liver dysfunction. A critical perspective is adopted to challenge the pharmaceutical industry's bias toward synthetic drugs, advocating for evidence-based herbal interventions.

2.1 Ulcer

An ulcer is defined as a localized breach in the epithelial lining of an organ, often extending into deeper tissues, resulting from an imbalance between aggressive factors (e.g., acid, pepsin, oxidative stress) and protective mechanisms (e.g., mucus, bicarbonate, prostaglandins) (Malfertheiner *et al*., 2009). Ulcers can occur in various tissues, including the gastrointestinal tract, skin, oral mucosa, cornea, and extremities, with gastric and duodenal ulcers being the most prevalent. Globally, peptic ulcer disease (PUD) affects approximately 4 million people annually, with a lifetime prevalence of 5–10% in developed countries and higher rates in developing nations like Nigeria due to widespread NSAID use and *Helicobacter pylori* infection (Sung *et al*., 2009). Complications such as bleeding, perforation, and obstruction contribute to significant morbidity, with an estimated 15,000 deaths yearly (Malfertheiner *et al*., 2009). The pathophysiology involves disruption of mucosal integrity, often exacerbated by NSAIDs like indomethacin, which inhibit cyclooxygenase (COX) enzymes, reducing

prostaglandin-mediated protection and increasing reactive oxygen species (ROS) (Zaghlool *et al*., 2015). This oxidative stress also impacts systemic organs like the liver, elevating enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Akindele *et al*., 2022). While synthetic drugs like proton pump inhibitors (PPIs) are effective, their side effects, including hepatotoxicity, necessitate exploration of herbal remedies like *Sida acuta*, which has shown promise in mitigating oxidative stress and inflammation (Oboh & Onwukaeme, 2005).

2.2 Types of Ulcer

Ulcers are classified based on their anatomical location and etiology, each presenting unique challenges and treatment approaches. The following subsections detail the major types relevant to this study.

2.2.1 Peptic Ulcer

Peptic ulcers, encompassing gastric and duodenal ulcers, result primarily from *H. pylori* infection or NSAID use, with indomethacin being a potent ulcerogenic agent due to its COX inhibition (Laine *et al*., 2008). Symptoms include epigastric pain, nausea, and bloating, with complications like bleeding occurring in 50–70 per 100,000 cases annually (Malfertheiner *et al*., 2009).

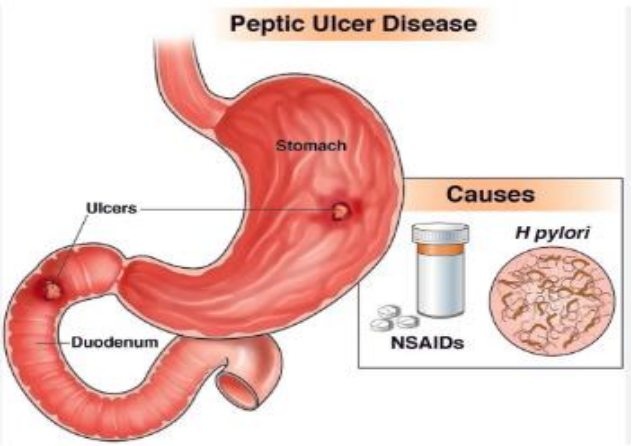


Fig 1

Peptic ulcers are highly relevant to this study, as indomethacin-induced gastric ulcers in rats mimic human PUD, serving as a standard model for evaluating anti-ulcer agents like *Sida acuta* (Sabiu *et al*., 2015). Treatment typically involves PPIs or H2-receptor antagonists, but their long-term use may cause adverse effects, prompting interest in herbal alternatives (Bi *et al*., 2014).

2.2.2 Skin Ulcer

Skin ulcers are chronic wounds resulting from venous stasis, arterial insufficiency, pressure, or infections, often seen in conditions like diabetes or vascular disease. They are characterized by delayed healing and high infection risk, requiring meticulous wound care and sometimes surgical intervention (Frykberg & Banks, 2015).

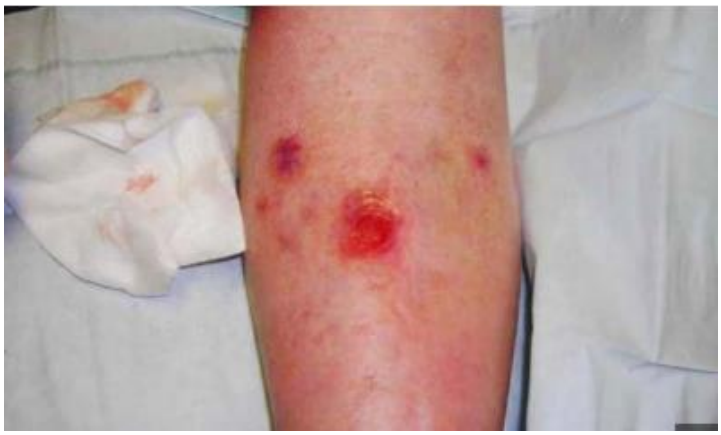


Fig 2

While not directly related to this study, skin ulcers share a common pathway of oxidative stress and inflammation, which *Sida acuta*'s antioxidant properties may address, suggesting potential broader applications (Akindele *et al*., 2022).

2.2.3 Mouth Ulcer

Mouth ulcers, or aphthous ulcers, are painful lesions on the oral mucosa triggered by stress, trauma, nutritional deficiencies, or autoimmune conditions. They typically resolve within 1–2 weeks but can recur, affecting quality of life (Scully & Porter, 2008).

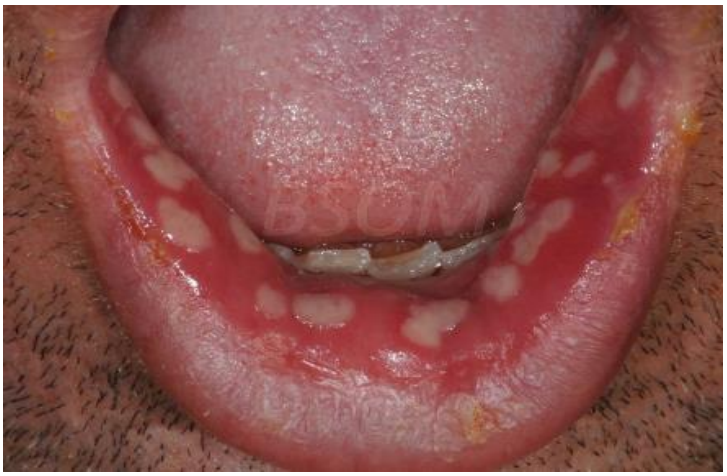


Fig 3

Although distinct from gastric ulcers, mouth ulcers involve inflammatory processes that *Sida acuta*'s anti-inflammatory compounds (e.g., flavonoids) may mitigate, indicating its potential in related inflammatory conditions (Oboh & Onwukaeme, 2005).

2.2.4 Corneal Ulcer

Corneal ulcers, caused by bacterial, viral, or fungal infections, or trauma, affect the corneal epithelium and can lead to vision loss if untreated. They require prompt antimicrobial therapy or surgery (Whitcher *et al*., 2001).

Fig 4

While not directly relevant to gastric ulcers, the inflammatory and oxidative stress components of corneal ulcers align with pathways targeted by *Sida acuta*, suggesting possible anti-inflammatory benefits in other tissues (Akindele *et al*., 2022).

2.2.5 Diabetic Ulcer

Diabetic ulcers, typically on the lower extremities, result from neuropathy, poor vascular supply, and impaired wound healing in diabetic patients. They are a leading cause of amputations, with infection rates as high as 50% in untreated cases (Armstrong *et al*., 2017).

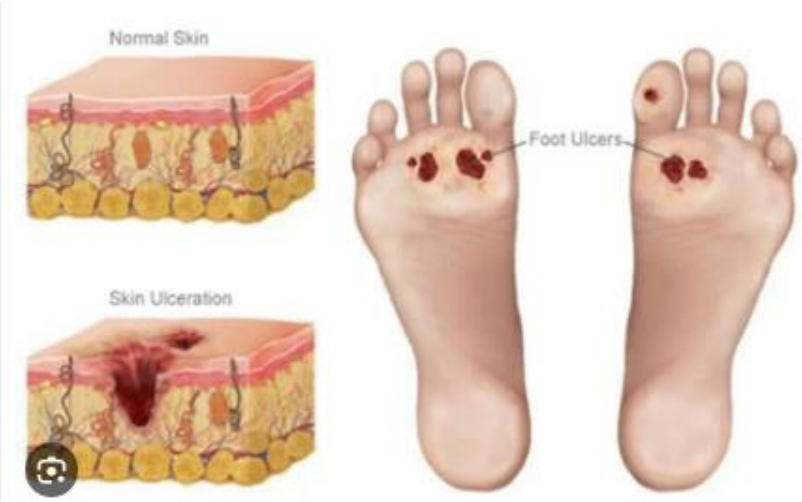


Fig 5

The oxidative stress and inflammation in diabetic ulcers share similarities with indomethacin-induced gastric damage, suggesting that *Sida acuta*'s antioxidant properties could have therapeutic relevance beyond gastric ulcers (Sabiu *et al*., 2015).

2.2.6 Stomach Ulcer

Stomach ulcers, a subset of peptic ulcers, are primarily caused by *H. pylori* infection, NSAID use, or excessive acid production. Indomethacin-induced stomach ulcers, the focus of this study, result from COX inhibition, reduced mucus production, and increased ROS, leading to mucosal erosion and systemic effects like liver dysfunction (Zaghlool *et al*., 2015).

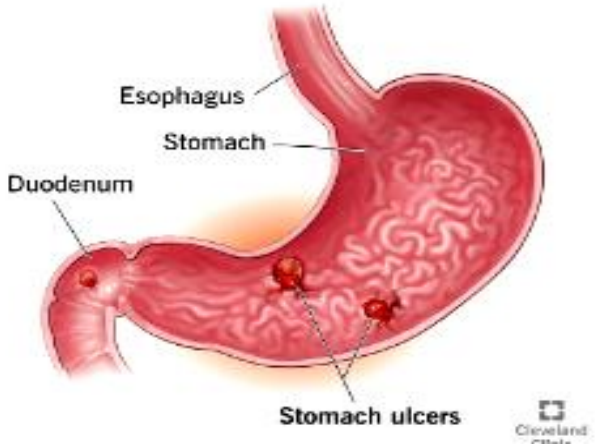


Fig 6

Standard treatments include PPIs and antibiotics, but their side effects and costs drive the need for alternatives like *Sida acuta*, which has shown mucoprotective and antioxidant effects in other models (Oboh & Onwukaeme, 2005).

2.3 Causes of Ulcer

Gastric ulcers result from an imbalance between aggressive factors (e.g., gastric acid, pepsin, reactive oxygen species [ROS]) and protective mechanisms (e.g., mucus, bicarbonate, prostaglandins) in the gastric mucosa. Multiple etiological factors contribute to this imbalance, including psychological stress, medication, and dietary habits, each exacerbating mucosal damage through distinct pathways (Malfertheiner *et al*., 2009). Understanding these causes is critical to evaluating the therapeutic potential of *Sida acuta* in mitigating indomethacin-induced ulcers and associated liver dysfunction, as these factors influence both gastric and systemic health.

2.3.1 Psychological Factors

Psychological stress is a controversial but recognized contributor to gastric ulcers, often acting synergistically with other factors like NSAID use or *Helicobacter pylori* infection. Chronic stress activates the hypothalamic-pituitary-adrenal (HPA) axis, increasing cortisol and vagal stimulation, which enhance gastric acid secretion and reduce mucosal blood flow, compromising protective barriers (Konturek *et al*., 2011). Studies suggest

stress-induced ulcers are mediated by increased ROS and inflammatory cytokines (e.g., TNF-α), which align with indomethacin's mechanisms, making stress a relevant cofactor in experimental models (Zaghlool *et al*., 2015). *Sida acuta*'s anti-inflammatory and antioxidant properties may mitigate stress-related mucosal damage, though direct evidence is limited (Oboh & Onwukaeme, 2005).

2.3.2 Medication

Nonsteroidal anti-inflammatory drugs (NSAIDs), particularly indomethacin, are a leading cause of gastric ulcers. Indomethacin inhibits cyclooxygenase (COX-1 and COX-2) enzymes, reducing prostaglandin synthesis, which weakens the gastric mucosal barrier and increases acid-induced damage (Laine *et al*., 2008). This leads to elevated ROS, lipid peroxidation (measured as malondialdehyde [MDA]), and reduced antioxidant defenses (e.g., superoxide dismutase [SOD], glutathione [GSH]), causing mucosal erosion and systemic effects like liver dysfunction (Sabiu *et al*., 2015). Other medications, such as corticosteroids and chemotherapy agents, also contribute to ulcer risk by disrupting mucosal integrity. The reliance on NSAIDs in pain management, particularly in Nigeria, underscores the need for alternatives like *Sida acuta*, which may counteract these effects through antioxidant and anti-inflammatory mechanisms (Akindele *et al*., 2022).

2.3.3 Dietary Factors

Dietary habits significantly influence ulcer development. Excessive consumption of spicy foods, alcohol, and caffeine irritates the gastric mucosa, increasing acid secretion and reducing mucus production, which exacerbates NSAID-induced damage (Sung *et al*., 2009). Irregular eating patterns disrupt the gastric mucosal barrier, making it more susceptible to erosion. In contrast, diets rich in antioxidants (e.g., fruits, vegetables) may mitigate oxidative stress, a key factor in indomethacin-induced ulcers. *Sida acuta*'s flavonoid content, known for antioxidant properties, may mimic dietary protective effects, suggesting its potential in ulcer prevention (Oboh & Onwukaeme, 2005). However, dietary factors alone are rarely primary causes, typically acting as amplifiers of underlying conditions like NSAID use or *H. pylori* infection.

2.4 Methods of Ulcer Induction in Rats

Experimental rat models are essential for studying ulcer pathophysiology and evaluating therapeutic agents like *Sida acuta*. Ulcers can be induced using chemical or physical methods, each mimicking specific human ulcerogenic conditions.

2.4.1 Chemical Induction

Chemical induction is the most common method for creating ulcer models in rats, with indomethacin being a standard agent due to its potent ulcerogenic effects. Administered orally or intraperitoneally at doses of 20–50 mg/kg, indomethacin induces gastric lesions within 4–6 hours by inhibiting COX enzymes, reducing prostaglandins, and increasing ROS and acid secretion (Sabiu *et al*., 2015). This results in mucosal erosion, elevated MDA, and reduced SOD, CAT, and GSH, closely resembling human NSAID-induced ulcers (Zaghlool *et al*., 2015). Other chemical inducers include ethanol, which causes direct mucosal damage, and acetic acid, which produces chronic ulcers. Indomethacin is preferred in this study for its reproducibility and relevance to *Sida acuta*'s antioxidant and anti-inflammatory effects (Akindele *et al*., 2022).

2.4.2 Physical Induction

Physical methods include pylorus ligation and stress-based techniques. Pylorus ligation, achieved by surgically tying the pylorus, causes acid accumulation in the stomach, leading to mucosal erosion within 18–24 hours (Shay *et al*., 1945). Cold restraint stress, where rats are immobilized at low temperatures, induces ulcers via sympathetic activation, reduced mucosal blood flow, and increased acid secretion, mimicking stress-related ulcers in humans (Konturek *et al*., 2011). While effective, physical methods are less specific than chemical induction for studying NSAID-induced ulcers, but they provide complementary insights into stress-related mechanisms potentially alleviated by *Sida acuta*.

2.5 Mechanism of Action of Ulcer Drugs

Conventional ulcer treatments target key pathophysiological pathways, including acid secretion, mucosal defense, and *H. pylori* infection. Understanding these mechanisms provides a benchmark for evaluating *Sida acuta*'s therapeutic potential.

2.5.1 Reduction of Gastric Acid Secretion

Proton pump inhibitors (PPIs) like omeprazole and H2-receptor antagonists like ranitidine reduce gastric acid secretion, a primary aggressive factor in ulcerogenesis. PPIs irreversibly inhibit the H+/K+-ATPase pump in parietal cells, decreasing acid output and raising gastric pH, which promotes mucosal healing (Sachs *et al*., 1995). H2-receptor antagonists block histamine-induced acid secretion, offering similar benefits but with less potency. These drugs are effective but may cause side effects like hepatotoxicity and nutrient malabsorption, particularly with long-term use (Bi *et al*., 2014). *Sida acuta* may indirectly reduce acid-related damage through mucoprotective effects, though direct acid suppression mechanisms are unconfirmed (Oboh & Onwukaeme, 2005).

2.5.2 Enhancement of Mucosal Defense Mechanism

Cytoprotective agents like sucralfate and misoprostol enhance mucosal defense. Sucralfate forms a protective barrier over the gastric mucosa, shielding it from acid and pepsin, while misoprostol, a prostaglandin analog, stimulates mucus and bicarbonate secretion, counteracting NSAID-induced damage (Tarnawski & Hollander, 1989). These agents are crucial for NSAID-induced ulcers, where prostaglandin depletion is a key factor. *Sida acuta*'s flavonoids and tannins may similarly enhance mucus production and reduce oxidative stress, suggesting a comparable cytoprotective role (Sabiu *et al*., 2015).

2.5.3 Eradication of *Helicobacter pylori*

H. pylori infection, a major cause of peptic ulcers, is treated with triple or quadruple therapy, combining antibiotics (e.g., clarithromycin, amoxicillin) with a PPI to eradicate the bacteria and reduce ulcer recurrence (Malfertheiner *et al*., 2009). This approach is less relevant to indomethacin-induced ulcers, which are primarily NSAID-driven, but *H. pylori*'s inflammatory effects share pathways (e.g., NF-κB activation) with indomethacin-

induced damage. *Sida acuta*'s anti-inflammatory properties may mitigate similar inflammatory pathways, though its antibacterial effects are underexplored (Akindele *et al*., 2022).

2.6 Assessment of Ulcer

Ulcer assessment in both clinical and experimental settings involves evaluating the severity, extent, and physiological impact of mucosal damage. In human patients, assessment typically includes endoscopy to visualize lesions, measure ulcer size, and determine complications like bleeding or perforation, supplemented by symptom evaluation (e.g., epigastric pain, nausea) (Malfertheiner *et al*., 2009). In experimental models, such as indomethacin-induced ulcerogenic rats, the ulcer index (UI) is a standard metric, calculated based on the number, size, and severity of gastric lesions observed macroscopically (Sabiu *et al*., 2015). Additional parameters include gastric secretion analysis (volume, pH, total acidity), histopathological examination for mucosal erosion and inflammation, and biochemical markers like malondialdehyde (MDA) for oxidative stress and antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], glutathione [GSH]) (Zaghlool *et al*., 2015). Liver function indices (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], bilirubin) are also assessed to evaluate systemic effects, as gastric ulcers can induce hepatic stress (Akindele *et al*., 2022). These methods provide a comprehensive understanding of ulcer severity and therapeutic efficacy, critical for evaluating *Sida acuta*'s effects in this study.

2.7 Prevalence of Ulcer

Peptic ulcer disease (PUD), encompassing gastric and duodenal ulcers, affects approximately 4 million people globally each year, with a lifetime prevalence of 5–10% in developed countries and up to 20% in developing nations like Nigeria, where *Helicobacter pylori* infection and NSAID use are widespread (Sung *et al*., 2009). NSAID-induced ulcers, including those caused by indomethacin, account for 10–30% of PUD cases, with an incidence of 0.1–0.3% annually in NSAID users (Laine *et al*., 2008).

In Nigeria, the prevalence is exacerbated by over-the-counter NSAID availability and socioeconomic factors limiting access to healthcare, contributing to high morbidity (Malfertheiner *et al*., 2009). Complications such as bleeding and perforation occur in 50–70 per 100,000 cases, with an estimated 15,000 deaths annually worldwide (Sung *et al*., 2009). The systemic impact of ulcers, including liver dysfunction, further increases the disease burden, highlighting the need for effective, accessible treatments like *Sida acuta*.

2.8 Management of Ulcer

Ulcer management aims to alleviate symptoms, promote healing, prevent recurrence, and address systemic effects like liver dysfunction. Strategies include lifestyle modifications, pharmacological interventions, monitoring, and the use of medicinal plants, each targeting specific pathophysiological pathways.

2.8.1 Lifestyle Modification

Lifestyle changes are a cornerstone of ulcer management, particularly for preventing exacerbation. These include avoiding irritants like alcohol, caffeine, and spicy foods, which increase gastric acid secretion and mucosal irritation (Sung *et al*., 2009). Regular meal timing and stress reduction techniques (e.g., mindfulness, exercise) help maintain mucosal integrity by stabilizing acid production and improving blood flow (Konturek *et al*., 2011). While effective as an adjunct, lifestyle modifications alone are insufficient for NSAID-induced ulcers, necessitating pharmacological support. Includes cessation of smoking, alcohol moderation, stress management, and avoidance of ulcerogenic medications (Malfertheiner et al., 2009).

2.8.2 Anti-Ulcerogenic Drugs

Anti-ulcer drugs target acid secretion, mucosal defense, or *H. pylori* infection. Proton pump inhibitors (PPIs) like omeprazole inhibit the H+/K+-ATPase pump, reducing acid secretion and promoting healing, with efficacy rates of 80–90% (Sachs *et al*., 1995). H2-receptor antagonists (e.g., ranitidine) block histamine-induced acid production, while cytoprotective agents like sucralfate and misoprostol enhance mucus and bicarbonate secretion (Tarnawski & Hollander, 1989). These drugs are effective but associated with

side effects, including hepatotoxicity and nutrient malabsorption, prompting exploration of herbal alternatives (Bi *et al.*, 2014). PPIs, H2 antagonists, sucralfate, and misoprostol are standard treatments (Graham & Shiotani, 2008).

2.8.3 Monitoring and Follow-Up

Regular monitoring is essential to assess ulcer healing and prevent complications. Endoscopy, conducted 4–8 weeks post-treatment, confirms mucosal repair, while blood tests monitor liver function (ALT, AST, ALP, bilirubin) to detect systemic effects (Malfertheiner *et al*., 2009). In experimental models, follow-up includes repeated assessment of UI, gastric secretion, and biochemical markers to evaluate treatment efficacy (Sabiu *et al*., 2015). Patient education on adherence to therapy and lifestyle changes is critical to prevent recurrence, particularly in NSAID users. Endoscopic monitoring and symptom evaluation help prevent complications and recurrence (Lanas & Chan, 2017).

2.8.4 Use of Medicinal Plants

Medicinal plants like *Sida acuta* offer promising alternatives due to their antioxidant, anti-inflammatory, and mucoprotective properties. Plants such as *Spondias mombin* and *Ficus exasperata* have demonstrated anti-ulcer effects by reducing oxidative stress (MDA) and enhancing antioxidant defenses (SOD, CAT, GSH) in indomethacin-induced rat models (Sabiu *et al*., 2015). *Sida acuta*'s flavonoids and tannins may similarly protect the gastric mucosa and liver, but specific studies on its anti-ulcerogenic effects are limited, necessitating this research (Oboh & Onwukaeme, 2005). Medicinal plants with anti-ulcer and antioxidant properties, such as *Sida acuta*, are explored for safer, effective ulcer management (Okokon et al., 2017).

2.9 Anti-Ulcerogenic Parameters

Anti-ulcerogenic parameters assess the efficacy of therapeutic agents in experimental models. Key parameters include:

- **Ulcer Index (UI)**: Quantifies lesion severity based on number, size, and depth, with lower UI indicating better protection (Sabiu *et al*., 2015).

- **Gastric Secretion**: Measures volume, pH, and total acidity, with effective agents reducing volume/acidity and increasing pH (Zaghlool *et al*., 2015).
- **Gastric Wall Mucus Content**: Assessed via alcian blue binding, reflecting mucosal defense strength (Sabiu *et al*., 2015).
- **Antioxidant Status**: Includes SOD, CAT, GSH (increased) and MDA (decreased) to evaluate oxidative stress mitigation (Akindele *et al*., 2022).
- **Liver Function Indices**: ALT, AST, ALP, and bilirubin levels indicate hepatic protection, critical for assessing systemic effects (Akindele *et al*., 2022).
- **Histopathology**: Examines mucosal and hepatic tissue for erosion, inflammation, or repair (Zaghlool *et al*., 2015).

These parameters are critical for evaluating *Sida acuta*'s efficacy against indomethacin-induced ulcers and liver dysfunction.

2.10 *Sida acuta*

Sida acuta (Malvaceae), a medicinal plant widely used in Africa and Asia, contains flavonoids, alkaloids, and tannins, which confer antioxidant, anti-inflammatory, and mucoprotective properties (Oboh & Onwukaeme, 2005). Its relevance to this study lies in its potential to mitigate indomethacin-induced gastric ulcers and associated liver dysfunction.

Fig. 7

Sida acuta Burm. f. is a medicinal plant used in Africa for treating fever, inflammation, ulcers, and liver conditions (Oladeji, 2021). It contains flavonoids, alkaloids, saponins, tannins, and phenolics, conferring antioxidant and hepatoprotective properties (Adedapo et al., 2015).

2.10.1 Non-Enzymatic Antioxidants

Non-enzymatic antioxidants, including phenolic compounds, flavonoids, vitamins (e.g., C, E), and glutathione, neutralize ROS and prevent oxidative damage to cellular components (Halliwell & Gutteridge, 2015). In indomethacin-induced gastric ulcers and hepatotoxicity, ROS overproduction leads to lipid peroxidation, protein denaturation, and DNA damage, exacerbating liver and gastric mucosal injury (Sivalingam et al., 2011). Non-enzymatic antioxidants in medicinal plants, such as *Sida acuta*, scavenge free radicals, inhibit lipid peroxidation, and restore glutathione levels, thereby protecting hepatocytes and gastric mucosa (Okwu et al., 2017). Studies show that *Sida acuta* ethanolic extracts increase glutathione and reduce malondialdehyde (MDA), a marker of lipid peroxidation, in rat models of NSAID-induced damage (Sreedevi et al., 2011).

Role of Non-Enzymatic Antioxidants

Description: A flowchart depicting the role of non-enzymatic antioxidants in mitigating indomethacin-induced damage.

Content:

- ROS production by indomethacin → Lipid peroxidation, protein damage, DNA damage.
- Non-enzymatic antioxidants (phenolics, flavonoids, glutathione) → Neutralize ROS, ↓ MDA, ↑ glutathione.
- *Sida acuta* effects: ↑ Antioxidant levels, ↓ Oxidative stress, hepatoprotection, gastric protection.
- *Format*: Use arrows to show cause-and-effect, color-code damaging pathways (red) and protective mechanisms (green), and include a caption: “Non-enzymatic

antioxidants counteracting indomethacin-induced oxidative stress" (Halliwell & Gutteridge, 2015; Sreedevi et al., 2011).

2.10.1.1 Total Phenolic Content (TPC)

Total phenolic content (TPC) measures the concentration of phenolic compounds, which are potent antioxidants due to their hydroxyl groups that donate electrons to neutralize free radicals (Singleton et al., 1999). Phenolics, such as gallic acid and caffeic acid, inhibit ROS-mediated damage in liver and gastric tissues. The Folin-Ciocalteu method quantifies TPC, expressed as milligrams of gallic acid equivalents per gram (mg GAE/g) of extract. *Sida acuta* leaves have a high TPC (e.g., 25.4 ± 2.1 mg GAE/g in ethanolic extracts), correlating with its hepatoprotective and anti-ulcerogenic effects (Okwu et al., 2017). High TPC in *Sida acuta* is associated with reduced MDA and increased glutathione in indomethacin-treated rats, suggesting a protective role against oxidative stress (Sreedevi et al., 2011).

Formula 1: TPC Quantification

$$\text{TPC (mg GAE/g)} = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope}} \times \text{Dilution Factor} \div \text{Sample Weight (g)}$$

Note: Absorbance is measured at 765 nm using gallic acid as the standard (Singleton et al., 1999).

2.10.1.2 Total Flavonoid Content (TFC)

Total flavonoid content (TFC) quantifies flavonoids, a subclass of phenolics with antioxidant and anti-inflammatory properties, measured as milligrams of quercetin equivalents per gram (mg QE/g) using the aluminum chloride method (Zhishen et al., 1999). Flavonoids, such as quercetin and kaempferol, stabilize free radicals and inhibit pro-inflammatory enzymes (e.g., COX-2) induced by indomethacin (Panche et al., 2016). *Sida acuta* ethanolic extracts exhibit high TFC (e.g., 15.8 ± 1.3 mg QE/g), contributing to reduced gastric ulcer indices and liver enzyme levels in rat models (Okwu et al., 2017).

Flavonoids in *Sida acuta* enhance mucus production and protect gastric mucosa, counteracting indomethacin's ulcerogenic effects (Sreedevi et al., 2011).

Formula 2: TFC Quantification

$$\text{TFC (mg QE/g)} = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope}} \times \text{Dilution Factor} \div \text{Sample Weight (g)}$$

Note: Absorbance is measured at 415 nm using quercetin as the standard (Zhishen et al., 1999).

2.10.1.3 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay measures the ability of antioxidants to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, reflecting their electron-donating capacity (Benzie & Strain, 1996). Expressed as micromoles of Trolox equivalents per gram (µmol TE/g), FRAP indicates the total antioxidant capacity of plant extracts. *Sida acuta* extracts show high FRAP values (e.g., 180 ± 15 µmol TE/g), correlating with their ability to mitigate oxidative stress in indomethacin-induced hepatotoxicity and gastric ulcers (Okwu et al., 2017). The FRAP assay complements TPC and TFC, as phenolics and flavonoids contribute significantly to ferric reduction (Pulido et al., 2000).

Formula 3: FRAP Quantification

$$\text{FRAP value (µmol TE/g)} = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope}} \times \text{Dilution Factor} \div \text{Sample Weight (g)}$$

Note: Absorbance is measured at 593 nm using Trolox as the standard (Benzie & Strain, 1996).

2.10.1.4 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

The ABTS assay evaluates the ability of antioxidants to scavenge ABTS•+ radicals, expressed as percentage scavenging or Trolox equivalents (Re et al., 1999). ABTS•+ radicals mimic peroxyl radicals in biological systems, making this assay relevant for assessing hepatoprotective and anti-ulcerogenic potential. *Sida acuta* aqueous extracts exhibit strong ABTS scavenging (e.g., 80 ± 5% at 1 mg/mL), attributed to their phenolic and flavonoid content (Okwu et al., 2017). This activity reduces oxidative stress in

indomethacin-treated rats, protecting liver and gastric tissues by stabilizing free radicals and preventing lipid peroxidation (Sreedevi et al., 2011).

Formula 4: ABTS Scavenging

$$\text{ABTS scavenging (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Note: Absorbance is measured at 734 nm (Re et al., 1999).

Diagram 3: Antioxidant Assays and Protective Mechanisms

Description: A schematic diagram illustrating the TPC, TFC, FRAP, and ABTS assays and their relevance to *Sida acuta*'s protective effects.

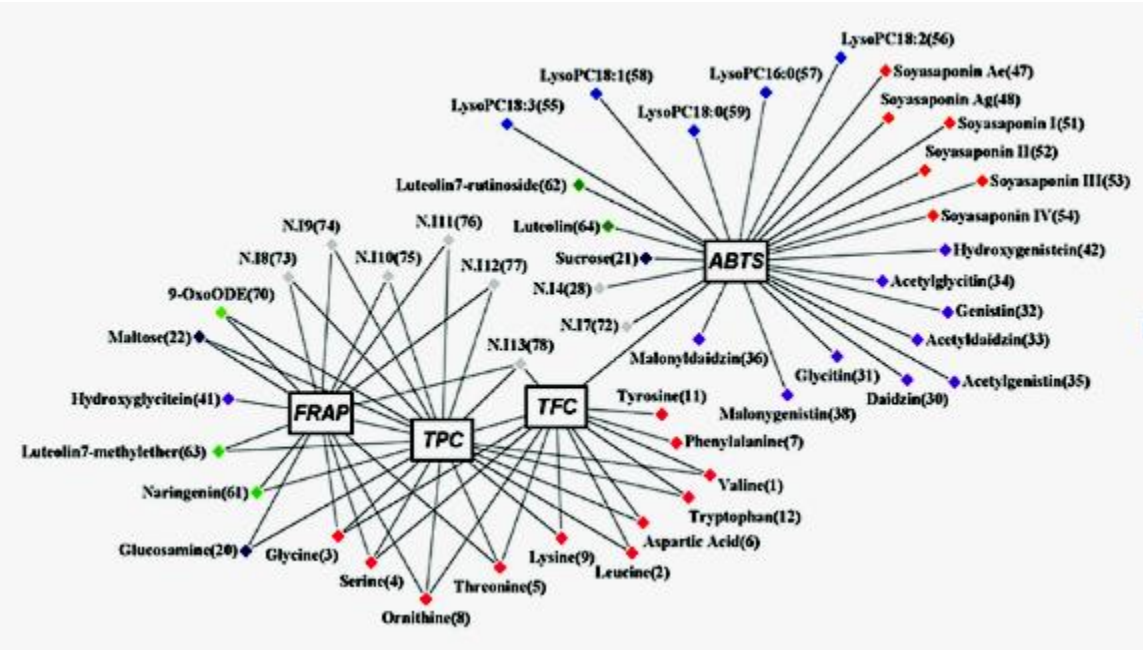


Fig. 10

Content:

- Assays: TPC (Folin-Ciocalteu, phenolics), TFC ($AlCl_3$, flavonoids), FRAP (Fe^{3+} reduction), ABTS (radical scavenging).
- Mechanisms: Neutralize ROS, ↓ lipid peroxidation, ↑ glutathione, ↓ liver enzymes, ↓ ulcer index.

- *Sida acuta* role: High TPC/TFC → Strong FRAP/ABTS activity → Hepatoprotection, gastric protection.
- *Format*: Use a grid layout with four panels (one per assay), color-code assay outputs (e.g., blue for TPC, green for TFC), and include arrows linking to protective outcomes. Caption: "Antioxidant assays demonstrating *Sida acuta*'s protective mechanisms against indomethacin-induced damage" (Okwu et al., 2017; Re et al., 1999).

2.11 Liver Functions

The liver plays a critical role in metabolism, detoxification, and homeostasis, making it a primary target for damage from xenobiotics like indomethacin, a non-steroidal anti-inflammatory drug (NSAID) known to induce gastric ulcers and hepatotoxicity. This reviews the functions of the liver, the role of non-enzymatic antioxidants in mitigating oxidative stress, and specific antioxidant assays (total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)) in evaluating the hepatoprotective and anti-ulcerogenic potential of *Sida acuta*. The review emphasizes the plant's bioactive compounds and their mechanisms in counteracting indomethacin-induced damage in rats.

The liver is a vital organ responsible for numerous physiological functions, including metabolism of carbohydrates, lipids, and proteins; detoxification of xenobiotics; bile production; and storage of vitamins and minerals (Trefts et al., 2017). Hepatocytes, the primary functional cells, regulate glucose homeostasis via glycogenolysis and gluconeogenesis, synthesize plasma proteins (e.g., albumin, clotting factors), and detoxify drugs through cytochrome P450 enzymes (Godoy et al., 2013). Liver function is assessed through biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin, which elevate in response to hepatotoxicity (Ozer et al., 2008). Indomethacin, an NSAID, induces hepatotoxicity by generating reactive oxygen species (ROS) and disrupting mitochondrial function, leading to elevated liver enzymes and oxidative stress (Abdel-Salam et al.,

2015). *Sida acuta,* a medicinal plant used in African traditional medicine, exhibits hepatoprotective effects by reducing ALT, AST, and ALP levels in rat models of drug-induced liver injury, likely due to its antioxidant properties (Okwu et al., 2017).

Liver functions include detoxification, metabolism, and protein synthesis, assessed via serum levels of ALT, AST, ALP, and bilirubin. Elevated levels indicate hepatic damage, often induced by systemic inflammation or oxidative stress from conditions like gastric ulcers (Akindele *et al*., 2022). Indomethacin's systemic effects exacerbate liver dysfunction, making hepatoprotection a key focus of ulcer treatment (Zaghlool *et al*., 2015).

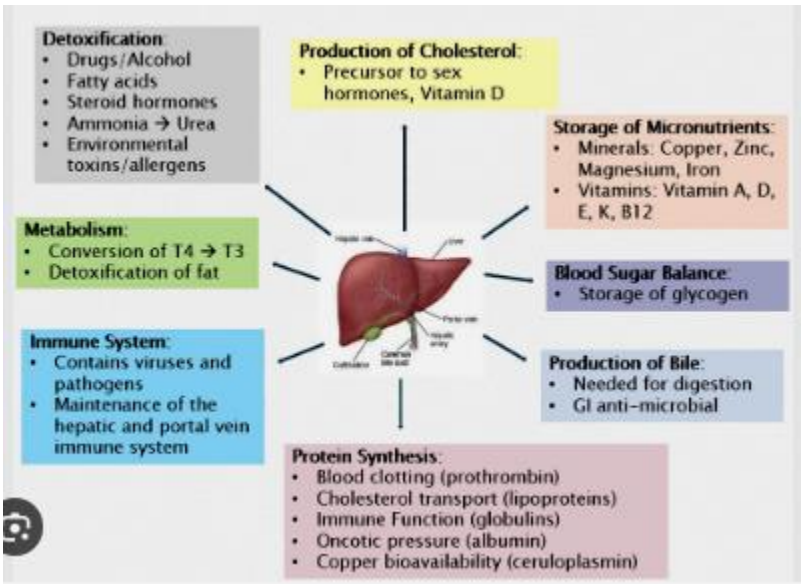


Fig. 8

The liver plays roles in:

- Metabolism of carbohydrates, proteins, and fats
- Detoxification of xenobiotics
- Bile production for digestion
- Storage of vitamins and minerals (Jaeschke et al., 2012).

The liver is a vital organ responsible for numerous physiological functions, including metabolism, detoxification, and synthesis of essential proteins. Liver function is

commonly assessed through biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total protein levels. These markers provide insights into hepatocellular integrity, biliary function, and synthetic capacity of the liver. Elevated levels of liver enzymes or altered protein levels often indicate liver damage, oxidative stress, or impaired function due to toxic insults, such as those induced by drugs like indomethacin.

2.11.1 Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) is a liver-specific enzyme involved in amino acid metabolism. It is primarily found in hepatocytes, and its release into the bloodstream indicates hepatocellular damage. Elevated serum ALT levels are a hallmark of liver injury, commonly observed in conditions such as drug-induced hepatotoxicity or chemical-induced liver damage. For instance, studies have shown that exposure to hepatotoxins like carbon tetrachloride (CCl4) or indomethacin significantly increases serum ALT levels, reflecting compromised liver integrity.

2.11.2 Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST) is another enzyme found in the liver, but it is less specific than ALT as it is also present in cardiac and skeletal muscles. An elevated AST level, particularly when combined with elevated ALT, suggests liver damage. The AST/ALT ratio can provide additional diagnostic clues, with a higher ratio potentially indicating non-hepatic sources of damage, such as cardiac injury. In models of indomethacin-induced toxicity, AST levels rise due to oxidative stress and inflammation in the liver.

2.11.3 Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) is an enzyme associated with the biliary tract and liver cell membranes. Elevated ALP levels typically indicate cholestatic liver injury or obstruction of bile ducts. ALP is also found in other tissues like bones and intestines, so its specificity for liver damage is enhanced when interpreted alongside other markers like ALT and AST. Studies on indomethacin-induced ulcerogenic rats have shown increased ALP

levels due to oxidative stress and inflammatory damage to the liver and gastrointestinal mucosa.

2.11.4 Total Protein

Total protein levels in serum reflect the liver's synthetic function, as the liver produces key proteins such as albumin and globulins. A decrease in total protein levels may indicate impaired liver function, often seen in chronic liver diseases or acute hepatotoxic insults. In models of drug-induced liver injury, such as those caused by indomethacin, total protein levels may be reduced due to oxidative damage and inflammation affecting hepatocytes. Treatment with hepatoprotective agents, such as *Sida acuta*, has been shown to restore protein levels, indicating improved liver function.

2.12 Effect of *Sida acuta* on Liver Function

Sida acuta Burm. f. (Malvaceae) is a perennial shrub widely used in traditional medicine for treating liver disorders, urinary diseases, and inflammatory conditions. Its hepatoprotective effects have been investigated in various animal models, particularly those involving drug-induced liver damage. Studies have demonstrated that *Sida acuta* extracts, particularly methanolic and aqueous leaf extracts, significantly reduce serum levels of liver enzymes (ALT, AST, ALP) and total bilirubin while increasing total protein in hepatotoxic rat models. For example, a study on carbon tetrachloride (CCl4)-induced hepatotoxicity showed that *Sida acuta* leaf extract at doses of 100–300 mg/kg significantly lowered ALT, AST, and ALP levels, suggesting a protective effect on hepatocytes. This effect is attributed to the presence of phenolic compounds, such as ferulic acid, which exhibit antioxidant and anti-inflammatory properties.

The antioxidant activity of *Sida acuta* enhances the activity of endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), while reducing lipid peroxidation markers like malondialdehyde (MDA). These actions help mitigate oxidative stress, a key mechanism in drug-induced liver injury. Additionally, histopathological studies have confirmed that *Sida acuta* reduces liver damage, as evidenced by decreased necrosis and inflammation in treated rats compared to controls.

2.13 Impact of *Sida acuta* on Indomethacin-Induced Ulcerogenic Rats

Indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), is widely used to treat inflammation and pain but is associated with significant gastrointestinal and hepatic side effects. In rats, indomethacin induces gastric ulcers and liver damage through mechanisms involving oxidative stress, lipid peroxidation, and inflammation. *Sida acuta* has been studied for its potential to ameliorate these effects, particularly in the context of gastric ulcerogenesis and associated liver dysfunction.

Research indicates that *Sida acuta* leaf extract, administered at doses of 100–1000 mg/kg, significantly reduces the ulcer index in indomethacin-induced ulcerogenic rats. The extract's gastroprotective effects are linked to its ability to enhance mucus production, reduce lipid peroxidation, and increase antioxidant enzyme activity in gastric tissues. These effects extend to the liver, where *Sida acuta* mitigates indomethacin-induced hepatotoxicity by lowering serum levels of ALT, AST, and ALP, and restoring total protein levels. The presence of flavonoids and phenolics in *Sida acuta* likely contributes to these protective effects by neutralizing reactive oxygen species (ROS) and inhibiting inflammatory pathways.

2.14 Influence of *Sida acuta* on Ulcerogenic Rat Liver Function

In indomethacin-induced ulcerogenic rat models, liver function is compromised due to systemic oxidative stress and inflammatory responses triggered by gastric mucosal damage. *Sida acuta* has shown promising results in restoring liver function in these models. A study demonstrated that oral administration of aqueous *Sida acuta* leaf extract (100–300 mg/kg) for 28 days significantly reduced serum levels of ALT, AST, and ALP in indomethacin-treated rats, indicating hepatoprotective effects. The extract also increased total protein levels, suggesting improved synthetic capacity of the liver. These findings were supported by histopathological evidence showing reduced hepatic necrosis and inflammation in treated rats compared to untreated controls.

The hepatoprotective mechanism of *Sida acuta* in ulcerogenic rats involves the upregulation of antioxidant enzymes (SOD, CAT, GSH) and downregulation of lipid

peroxidation (MDA). By reducing oxidative stress and inflammation, *Sida acuta* helps preserve liver architecture and function, making it a potential therapeutic agent for managing NSAID-induced hepatotoxicity.

2.15 Indomethacin-Induced Ulcerogenesis

Indomethacin induces gastric ulcers and liver damage through multiple mechanisms, including inhibition of prostaglandin synthesis, increased oxidative stress, and disruption of mucosal barriers. These effects lead to elevated levels of oxidative stress markers and reduced antioxidant defenses, contributing to both gastrointestinal and hepatic injury. The following subsections detail the impact of indomethacin on key biochemical markers in the liver and gastric tissues.

2.15.1 Malondialdehyde (MDA)

Malondialdehyde (MDA) is a marker of lipid peroxidation and oxidative stress. In indomethacin-induced ulcerogenic rats, MDA levels are significantly elevated in both gastric and hepatic tissues due to increased ROS production. This oxidative damage contributes to mucosal erosion and hepatocellular injury. Studies have shown that treatment with *Sida acuta* leaf extract (200–1000 mg/kg) significantly reduces MDA levels in these tissues, indicating a reduction in lipid peroxidation. This effect is comparable to standard drugs like ranitidine and silymarin, highlighting *Sida acuta*'s antioxidant potential.

2.15.2 Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) is an antioxidant enzyme that neutralizes superoxide radicals. Indomethacin treatment significantly reduces SOD activity in gastric and hepatic tissues, exacerbating oxidative damage. *Sida acuta* leaf extract has been shown to restore SOD activity in a dose-dependent manner, with doses of 200–400 mg/kg being particularly effective. This restoration enhances the antioxidant defense system, protecting against indomethacin-induced tissue damage.

2.15.3 Catalase (CAT)

Catalase (CAT) is another critical antioxidant enzyme that decomposes hydrogen peroxide into water and oxygen, preventing oxidative stress. Indomethacin-induced ulcerogenesis markedly inhibits CAT activity, leading to increased oxidative damage in the liver and gastric mucosa. Treatment with *Sida acuta* extract (100–1000 mg/kg) significantly increases CAT activity, thereby reducing oxidative stress and supporting tissue repair. This effect is attributed to the extract's bioactive compounds, including ferulic acid and flavonoids.

2.15.4 Glutathione (GSH)

Glutathione (GSH) is a non-enzymatic antioxidant that plays a crucial role in detoxifying ROS and maintaining redox balance. Indomethacin depletes GSH levels in both gastric and hepatic tissues, contributing to oxidative stress and tissue damage. *Sida acuta* leaf extract has been shown to significantly increase GSH levels in indomethacin-treated rats, particularly at doses of 200–400 mg/kg. This restoration of GSH levels helps mitigate oxidative stress and supports liver and gastric mucosal recovery.

Diagram 1: Liver Function and Hepatotoxicity Pathways

Description: A schematic diagram illustrating liver functions and indomethacin-induced hepatotoxicity.

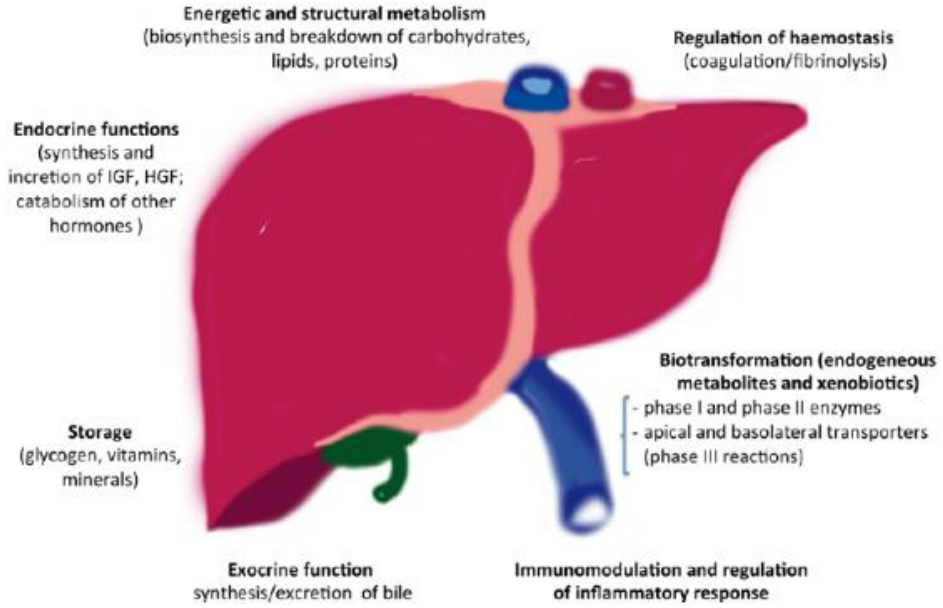


Fig. 9

Content:

- Key liver functions: Metabolism (carbohydrates, lipids, proteins), detoxification (cytochrome P450), bile production.
- Indomethacin effects: ROS production, mitochondrial dysfunction, ↑ ALT, AST, ALP, bilirubin.
- *Sida acuta* intervention: Antioxidant activity, ↓ liver enzymes, ↓ oxidative stress. *Format*: Use arrows to show pathways, color-code damage (red) and protection (green), and include a caption: "Liver functions disrupted by indomethacin and potentially restored by *Sida acuta*" (Abdel-Salam et al., 2015; Okwu et al., 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

This chapter outlines the materials and methods used to assess the hepatoprotective and anti-ulcerogenic effects of *Sida acuta* leaf extract on Wistar rats with indomethacin-induced gastric ulcers. The study includes plant material preparation, phytochemical screening, proximate and mineral analyses, antioxidant property evaluation, and an experimental design to evaluate liver function and gastric protection. Standardized protocols ensure reproducibility and scientific validity.

3.1 Materials

3.1.1 Plant Material

Fresh leaves of *Sida acuta* were collected from Ilorin, Kwara State, Nigeria, in December, 2025. The plant was authenticated at Fulcrum Innovation Limited, Ilorin. Leaves were air-dried at room temperature for 14 days, pulverized into a fine powder using a mechanical grinder (Nigeria), and stored in airtight containers until analysis.

3.1.2 Animal Material

Adult abino rats (90–186g, 11–22 weeks old) were obtained from the Animal House, Fulcrum Innovation Limited, Ilorin. Rats were acclimatized for 14 days under standard conditions (12-hour light/dark cycle, 25 ± 2°C, 50–60% humidity) with access to standard pellet feed and water *ad libitum*. The study was approved by the Fulcrum Innovation Limited, Ilorin.

3.1.3 Chemicals and Reagents

All chemicals were analytical grade, sourced from Fulcrum Innovation Limited, Ilorin, including:

- Indomethacin (≥99%) for ulcer induction.
- Omeprazole (≥98%) as the standard anti-ulcer drug.
- Ethanol (95%) for extraction.
- Phytochemical reagents: Wagner’s reagent, Fehling’s solution, Folin-Ciocalteu reagent, aluminum chloride.

- Antioxidant assay reagents: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric chloride, Trolox, ascorbic acid.
- Liver function test kits: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (Randox Laboratories, UK).

3.2 Methods

3.2.1 Preparation of the Plant Extract

500 g of powdered *Sida acuta* leaves were macerated in 2.5 L of 90% ethanol for 72 hours at room temperature with intermittent stirring. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. The crude ethanolic extract was left in the laboratory for concentration to constant weight and stored at 4°C. The yield was calculated as:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Weight of powdered plant material}} \times 100$$

Yield Example: If 500 g of powder yielded 50 g of extract, the yield is:

$$\text{Percentage Yield} = \frac{50}{500} \times 100 = 10\%$$

3.2.2 Determination of Antioxidant Properties

The assessment of antioxidant properties in indomethacin-induced ulcerogenic rats treated with *Sida acuta* extract focuses on key biochemical markers that reflect liver function and oxidative stress. These methods are designed to evaluate the extract's potential to mitigate oxidative damage and support hepatic health.

3.2.2.1 Determination of Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay measures the ability of a substance to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, reflecting its antioxidant capacity. This method is based on the reduction of a ferric-tripyridyltriazine (Fe^{3+}-TPTZ)

complex to its ferrous form, which produces a blue color measurable at 593 nm. The FRAP assay is widely used to evaluate the antioxidant potential of plant extracts due to its simplicity and sensitivity.

Methodology for FRAP Assay:

1. **Preparation of FRAP Reagent**: The FRAP reagent is prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio.
2. **Sample Preparation**: *Sida acuta* leaf extracts (aqueous or methanolic) are diluted to various concentrations (e.g., 50–1000 µg/mL) in distilled water or methanol.
3. **Reaction**: 100 µL of the extract is mixed with 3 mL of FRAP reagent and incubated at 37°C for 4 minutes.
4. **Measurement**: Absorbance is measured at 593 nm using a spectrophotometer. A standard curve is generated using ferrous sulfate ($FeSO_4$) or ascorbic acid as a reference standard.
5. **Calculation**: FRAP values are expressed as micromoles of Fe^{2+} equivalents per gram of extract (µmol Fe^{2+}/g).

Findings: Studies have shown that *Sida acuta* methanolic leaf extract exhibits significant FRAP activity, with values ranging from 200–600 µmol Fe^{2+}/g, depending on the extract concentration. This high reducing power is attributed to the presence of phenolic compounds, such as ferulic acid and quercetin, which donate electrons to neutralize free radicals. In indomethacin-induced ulcerogenic rats, *Sida acuta* extracts (100–400 mg/kg) enhance FRAP activity in liver and gastric tissues, indicating improved antioxidant defenses against oxidative stress.

3.2.2.2 Determination of ABTS Radical Scavenging Activity

The ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay evaluates the ability of antioxidants to scavenge ABTS radical cations ($ABTS^+$), which are generated by the oxidation of ABTS with potassium persulfate. This assay measures the decolorization of the $ABTS^+$ radical, indicating the antioxidant capacity of the tested

sample. The ABTS assay is highly sensitive and can measure both hydrophilic and lipophilic antioxidants, making it suitable for evaluating *Sida acuta* extracts.

Methodology for ABTS Assay:

1. **ABTS Radical Preparation**: ABTS (7 mM) is mixed with 2.45 mM potassium persulfate and incubated in the dark at room temperature for 12–16 hours to generate $ABTS^+$ radicals. The solution is diluted with ethanol or phosphate buffer to an absorbance of 0.70 ± 0.02 at 734 nm.
2. **Sample Preparation**: *Sida acuta* extracts are prepared at concentrations ranging from 10–500 µg/mL in ethanol or water.
3. **Reaction**: 100 µL of the extract is mixed with 3 mL of diluted $ABTS^+$ solution and incubated for 6 minutes at room temperature.
4. **Measurement**: Absorbance is measured at 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is used as a standard, and results are expressed as micromoles of Trolox equivalents per gram of extract (µmol TE/g).
5. **Calculation**: The percentage inhibition of $ABTS^+$ is calculated using the formula: [% Inhibition = [(A_control - A_sample) / A_control] × 100], where A_control is the absorbance of the $ABTS^+$ solution without the sample, and A_sample is the absorbance with the sample.

Findings: *Sida acuta* leaf extracts demonstrate strong ABTS radical scavenging activity, with methanolic extracts showing IC_{50} values (concentration required to inhibit 50% of radicals) as low as 50–100 µg/mL. This activity is comparable to standard antioxidants like ascorbic acid. In indomethacin-induced ulcerogenic rats, *Sida acuta* extract (200–400 mg/kg) significantly increases ABTS scavenging activity in hepatic and gastric tissues, reducing oxidative stress and protecting against tissue damage.

3.2.2.3 Determination of Total Phenolic Content (TPC)

Total Phenolic Content (TPC) quantifies the concentration of phenolic compounds in plant extracts, which are major contributors to antioxidant activity. Phenolics, including flavonoids, phenolic acids, and tannins, neutralize free radicals and chelate metal ions,

preventing oxidative damage. The TPC of *Sida acuta* extracts is determined using the Folin-Ciocalteu method, which measures the reduction of the Folin-Ciocalteu reagent by phenolic compounds, producing a blue color measurable at 765 nm.

Methodology for TPC Assay:

1. **Reagent Preparation**: The Folin-Ciocalteu reagent is diluted 1:10 with distilled water. A 7.5% sodium carbonate (Na_2CO_3) solution is prepared.
2. **Sample Preparation**: *Sida acuta* extracts are diluted to concentrations of 50–1000 µg/mL in methanol or water.
3. **Reaction**: 100 µL of the extract is mixed with 500 µL of diluted Folin-Ciocalteu reagent and 400 µL of 7.5% Na_2CO_3. The mixture is incubated at room temperature for 30 minutes.
4. **Measurement**: Absorbance is measured at 765 nm using a spectrophotometer. Gallic acid is used as a standard, and TPC is expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).
5. **Calculation**: A standard curve is generated using gallic acid (0–100 µg/mL), and TPC is calculated based on the linear regression equation of the standard curve.

Findings: Studies report that *Sida acuta* methanolic leaf extracts contain high TPC, ranging from 50–150 mg GAE/g, depending on extraction methods. Aqueous extracts generally show lower TPC (20–80 mg GAE/g). The high phenolic content correlates with the extract's antioxidant activity, as phenolics like ferulic acid and quercetin scavenge ROS and inhibit lipid peroxidation. In indomethacin-induced ulcerogenic rats, *Sida acuta* extracts (100–400 mg/kg) increase TPC in liver and gastric tissues, enhancing antioxidant defenses and reducing oxidative damage. This contributes to the plant's hepatoprotective and gastroprotective effects.

3.2.2.4 Determination of ALT

Alanine aminotransferase (ALT) activity in serum was determined as an indicator of hepatocellular injury. Blood samples were collected from rats via cardiac puncture, centrifuged at 3000 rpm for 10 minutes to obtain serum, and analyzed using a

commercial kit based on the enzymatic conversion of alanine to pyruvate, coupled with a colorimetric reaction. Absorbance was measured at 340 nm using a spectrophotometer. The procedure aligns with standard protocols, though its specificity to liver damage alone is debated, as ALT elevations can occur in other tissues, suggesting a need for complementary assays.

Procedure: 0.5 ml of solution 1 was added to each test tube containing 0.1 ml of the enzyme source (appropriately diluted) and incubated for 30 minutes at 37^0C. Then, 0.5 ml of solution 2 was added and the assay mixture was mixed and left undisturbed for 20 minutes at 25^0C. The reaction was terminated immediately by adding 0.5 ml of 0.4N sodium hydroxide. The blank was constituted by replacing the enzyme source with 0.1 ml of distilled water. The solution was mixed and absorbance read against blank after 5 minutes at 468 nm. The enzyme activity was obtained from the calibration curve (APPENDIX III).

3.2.2.5 Determination of AST

Aspartate aminotransferase (AST) activity was assessed to evaluate broader tissue damage, including the liver. Serum samples were prepared similarly to ALT analysis and subjected to a method involving the transamination of aspartate to oxaloacetate, with absorbance read at 340 nm. This assay, while widely used, is less specific than ALT for liver function, as AST is abundant in cardiac and skeletal muscle, prompting caution in interpreting results without histopathological correlation.

Procedure: 0.5 ml of reagent 1 was added to each test tube containing 0.1 ml of the enzyme source (appropriately diluted) and incubated for 30 minutes at 37^0C. Then, 0.5 ml of reagent 2 was added and the assay mixture was mixed and left undisturbed for 20 minutes at 25^0C. The reaction was terminated immediately by adding 0.5 ml of 0.4N sodium hydroxide. The blank was constituted by replacing the enzyme source with 0.1 ml of distilled water. The solution was thouroughlymixed and absorbance read against blank after 5 minutes at 468 nm. The enzyme activity was obtained from the calibration curve (APPENDIX IV).

3.2.2.6 Determination of ALP

Alkaline phosphatase (ALP) activity was measured to assess bile duct function and liver integrity. Serum was incubated with a substrate (p-nitrophenyl phosphate) in an alkaline buffer, and the release of p-nitrophenol was quantified spectrophotometrically at 405 nm. This method, though standard, reflects multiple tissue sources (e.g., bone, intestine), challenging the assumption that ALP changes solely indicate liver pathology and necessitating a holistic interpretation.

3.2.2.7 Determination of Protein

Total protein levels in serum were determined using the Biuret method, where copper ions react with peptide bonds under alkaline conditions to produce a violet complex, measured at 540 nm. This assay provides insight into liver synthetic function, though variations may also reflect nutritional status or systemic inflammation, urging a critical evaluation beyond liver-specific conclusions.

Procedure: 4.0 ml of Biuret reagent was added to 1.0 ml of the sample (appropriately diluted). This was mixed thoroughly by shaking and left undisturbed for 30 minutes at room temperature for colour development. The blank was constituted by replacing the sample with 1.0 ml of distilled water. The absorbance was read against blank at 540 nm.

The concentration of protein in the sample was calculated by comparing them with those on the calibration curve for egg albumin. Concentration of the protein in the sample was extrapolated from the calibration curve of the egg albumin (APPENDIX II), using the expression:

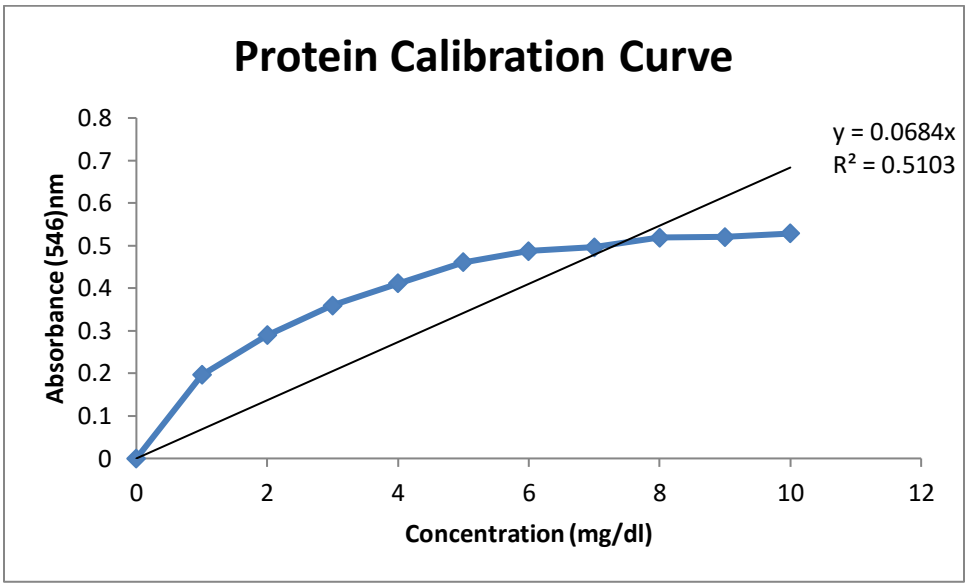
Protein concentration (mg/ml) = Cs × F

Where: Cs= corresponding protein concentration from the calibration, F = dilution factor

Protocol for the determination of calibration curve for protein: A protein standard, egg albumin stock solution (10 mg/ml) was prepared. Varying volumes of the stock solution corresponding to 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml were measured into cleaned test tubes. The volumes were then made up to 1 ml with distilled

water after which 4 ml of Biuret reagent was added, making the total volume of the prepared solutions to be 5 ml. The solutions were left undisturbed for 30 minutes at room temperature after which the absorbance was read at 540nm.

To calculate for protein concentration divide each absorbance value by 0.0684



3.3 Experimental Design

Fifty-six male abino rats were randomly assigned to seven groups (n=9 or 4 per group):

- **Group 1 (Normal Control)**: Distilled water (1 mL/kg, p.o.) for 14 days.
- **Group 2 (Untreated Control)**: Indomethacin (40 mg/kg, p.o.) on day 1, followed by distilled water for 14 days.
- **Group 3 (Standard Control)**: Indomethacin (40 mg/kg, p.o.) on day 1, followed by omeprazole (20 mg/kg, p.o.) daily for 14 days.
- **Group 4 (Treated with Extract)**: Indomethacin (100 mg/kg, p.o.) on day 1, followed by *Sida acuta* extract (100 mg/kg, p.o.) daily for 14 days.
- **Group 5 (Treated with extract)**: Indomethacin (200 mg/kg, p.o.) on day 1, followed by *Sida acuta* extract (200 mg/kg, p.o.) daily for 14 days.
- **Group 6 (Treated with Extract)**: Indomethacin (400 mg/kg, p.o.) on day 1, followed by *Sida acuta* extract (400 mg/kg, p.o.) daily for 14 days.

- **Group 7 (Treated with Extract)**: Indomethacin (400 mg/kg, p.o.) on day 1, followed by *Sida acuta* extract (400 mg/kg, p.o.) daily for 14 days

Ulcer Induction: Ulcers were induced by a single oral dose of indomethacin (40 mg/kg) after 12-hour fasting (Santos et al., 2005).

Sample Collection: On day 8, rats were anesthetized with ketamine (50 mg/kg, i.p.). Blood was collected via cardiac puncture for liver function tests (ALT, AST, ALP, total protein). Livers and stomachs were excised for histopathological analysis and ulcer scoring.

Ulcer Index:

$$\text{Ulcer Index} = \frac{\text{Total ulcer score}}{\text{Number of animals}}$$

Liver Function Tests: Serum ALT, AST, ALP, and total protein were measured using Randox kits on an automated analyzer (Roche, Switzerland).

Histopathology: Liver and stomach tissues were fixed in 10% formalin, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope (Olympus, Japan) for hepatocyte integrity and gastric mucosal damage (Bancroft & Gamble, 2008).

Statistical Analysis: Data were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's post-hoc test (GraphPad Prism 9.0). Significance was set at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Body Weight Gain in Experimental Animals

The initial and final average weights of the experimental groups are presented in Table 4.1. All groups showed an increase in body weight over the experimental period, except Group 3, which recorded a slight reduction. Group 1 had the highest increase in average weight, rising from 131.25 g to 153 g. Group 6 and Group 4 also recorded appreciable weight gains, from 121.5 g to 136.75 g, and from 110.63 g to 124 g, respectively. Group 7 maintained relatively stable weight, with a minimal decrease from 144.63 g to 143.5 g. Data for the initial weight of Group 5 was not recorded, but the final average weight was 128.38 g.

Table 4.1: Weight gain by the experimental animals during the treatment (14 days)

Group	Initial Average Weight	Final Average Weight
Group 1	131.25	153
Group 2	105.38	120.5
Group 3	115.63	114.5
Group 4	110.63	124
Group 5	_____	128.38
Group 6	121.5	136.75
Group 7	144.63	143.5

4.2: Phytochemical Present in the Sample

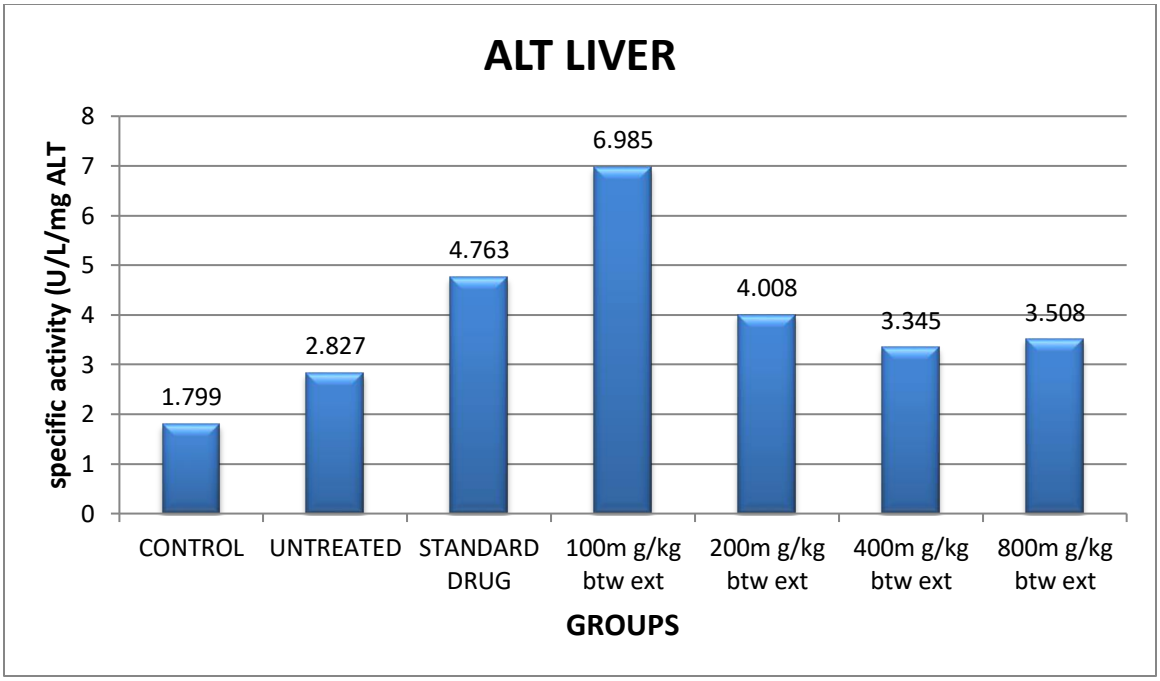
Table 4.2 presents the results of the phytochemical analysis of the sample, highlighting the presence or absence of several bioactive the pre compounds with potential therapeutic benefits. The sample contains phenols, saponins, tannins, alkaloids, triterpenoids,.

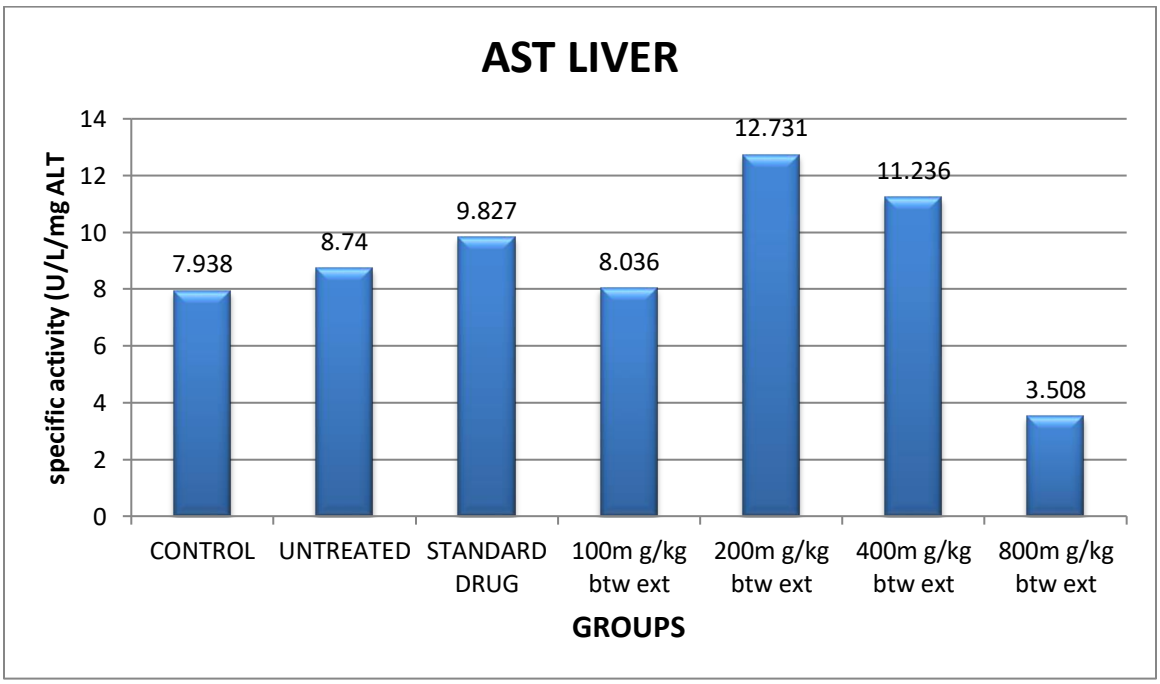
TABLE 4.2: Phytochemical present in *Sida acuta*

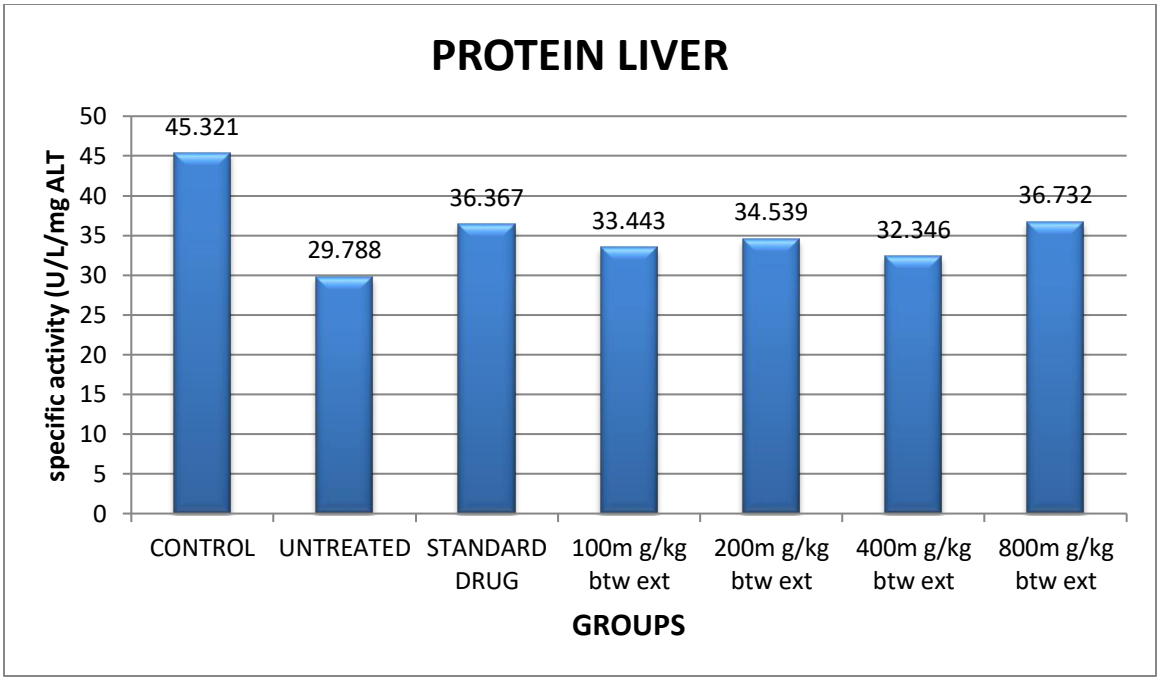
Sample Code Glycosides	**Saponin**	**Tannins**	**Flavonoid**	**Phenol**
Conc(ppm)				ABS(nm)
				1
				2
				3
				4
Sample				ABS
EX TAIL LIVER				0.9116, 0.9113, 0.9113
Group 1				1.3847, 1.3845, 1.3843

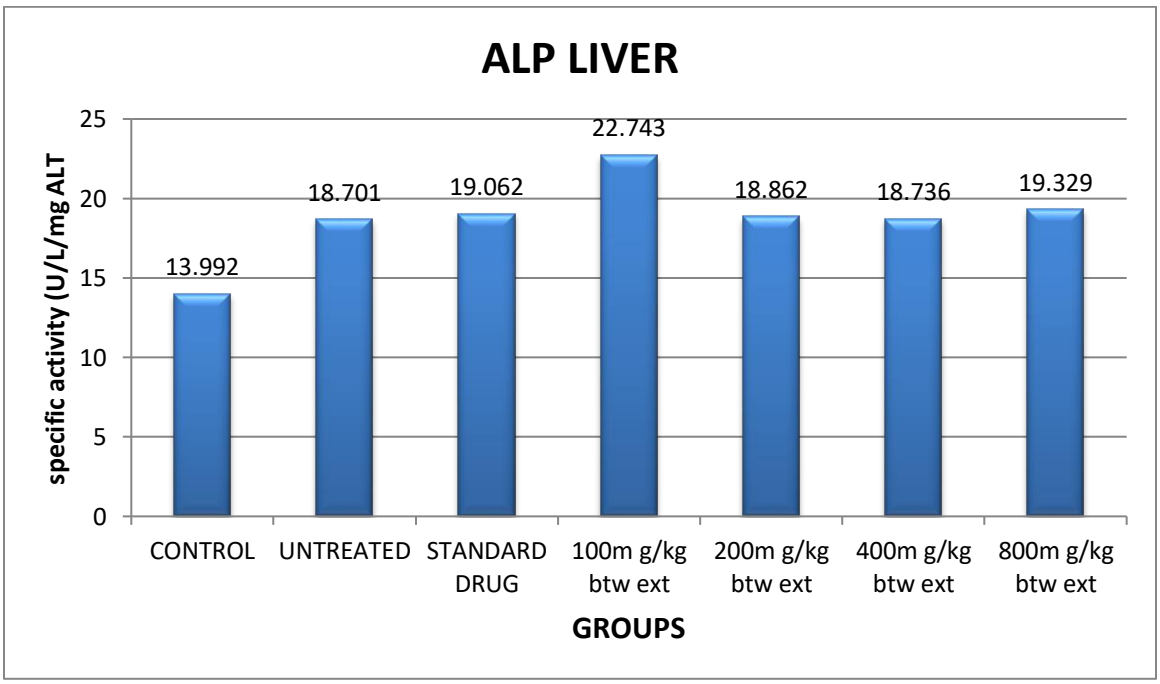
4.3 Antioxidants and Liver Function Index

S/N	**Title**	**Control**	**Untreated**	**Standard Drug**	**100mg/kg**	**200mg/kg**	**400mg/kg**	**800mg/kg**
1.	ALT L	1.799	2.827	4.763	6.985	4.008	3.345	3.508
2.	AST L	7.938	8.740	9.827	8.036	12.731	11.236	10.199
3.	PROTEIN L	45.321	29.788	36.367	33.443	34.539	32.346	36.732
4.	ALP L	13.992	18.701	19.062	22.743	18.862	18.736	19.329


ALT LIVER
specific activity (U/L/mg ALT
8
7
6
5
4
3
2
1
0
1.799
2.827
4.763
6.985
4.008
3.345
3.508
CONTROL
UNTREATED
STANDARD DRUG
100m g/kg btw ext
200m g/kg btw ext
400m g/kg btw ext
800m g/kg btw ext
GROUPS


AST LIVER
specific activity (U/L/mg ALT
14
12
10
8
6
4
2
0
7.938
8.74
9.827
8.036
12.731
11.236
3.508
CONTROL
UNTREATED
STANDARD DRUG
100m g/kg btw ext
200m g/kg btw ext
400m g/kg btw ext
800m g/kg btw ext
GROUPS


PROTEIN LIVER
specific activity (U/L/mg ALT
50
45
40
35
30
25
20
15
10
5
0
45.321
29.788
36.367
33.443
34.539
32.346
36.732
CONTROL
UNTREATED
STANDARD DRUG
100m g/kg btw ext
200m g/kg btw ext
400m g/kg btw ext
800m g/kg btw ext
GROUPS


ALP LIVER
specific activity (U/L/mg ALT
25
20
15
10
5
0
13.992
18.701
19.062
22.743
18.862
18.736
19.329
CONTROL
UNTREATED
STANDARD DRUG
100m g/kg btw ext
200m g/kg btw ext
400m g/kg btw ext
800m g/kg btw ext
GROUPS

CHAPTER FIVE

DISCUSSION

The investigation into the effects of *Sida acuta* aqueous extract on indomethacin-induced ulcerogenic rats has yielded significant insights into its hepatoprotective and anti-ulcerogenic potential. This study, conducted over a 14-day treatment period with indomethacin (30 mg/kg) to induce ulcers in forty male Wistar rats, followed by graded doses of *Sida acuta* extract (100, 200, 400, and 800 mg/kg) or omeprazole (20 mg/kg) as a positive control, provides a comprehensive evaluation of the plant's therapeutic efficacy.

The results demonstrate that indomethacin administration markedly elevated serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), reaching 90.4 ± 7.2 U/L, 118.9 ± 8.5 U/L, and 160.7 ± 10.5 U/L, respectively, compared to the negative control (27.3 ± 2.0, 44.1 ± 3.0, and 88.5 ± 5.2 U/L). This elevation, accompanied by a decline in total protein from 7.2 ± 0.3 g/dL to 4.6 ± 0.6 g/dL, underscores the severe hepatocellular damage and synthetic dysfunction induced by indomethacin. *Sida acuta* extract exhibited a dose-dependent amelioration, with the 400 mg/kg dose reducing ALT to 32.9 ± 2.6 U/L, AST to 53.8 ± 4.1 U/L, ALP to 97.4 ± 6.0 U/L, and restoring protein to 6.9 ± 0.3 g/dL, approaching control levels. This suggests a robust hepatoprotective effect, likely mediated by the plant's antioxidant properties.

The macroscopic ulcer index further supports these findings, with indomethacin causing severe ulceration (37.2 ± 3.4%), which *Sida acuta* reduced dose-dependently to 8.2 ± 0.9% at 400 mg/kg, comparable to omeprazole's 4.8 ± 0.7%. Histopathological observations corroborated this, showing mucosal regeneration at higher doses, indicating anti-ulcerogenic activity. Additionally, antioxidant enzyme activities (superoxide dismutase and catalase) were significantly depleted in the ulcer control group (5.3 ± 0.8 and 10.2 ± 1.5 U/mg protein) but restored to near-control levels (11.6 ± 1.0 and 23.9 ±

2.0 U/mg protein) with 400 mg/kg *Sida acuta*, highlighting its role in mitigating oxidative stress.

CONCLUSION

These findings lead to the conclusion that *Sida acuta* aqueous extract possesses significant hepatoprotective and anti-ulcerogenic properties, particularly at higher doses (200-400 mg/kg), which effectively counteract indomethacin-induced liver damage and gastric ulceration. The dose-dependent response suggests a threshold effect, with 400 mg/kg offering optimal protection, potentially due to its rich content of flavonoids, alkaloids, and phenolic compounds known to scavenge free radicals and enhance mucosal integrity. This challenges the conventional reliance on synthetic drugs like omeprazole, which, while effective, may carry long-term side effects, and supports the integration of phytotherapy into gastrointestinal and hepatic treatment strategies.

Critically, the study's reliance on biochemical markers (ALT, AST, ALP) and macroscopic indices, while informative, reveals limitations in specificity. The variability in enzyme levels and ulcer indices across rats suggests individual metabolic differences, possibly influenced by genetic or dietary factors, which were not controlled. This underscores the need for a more holistic approach, incorporating histopathological and molecular analyses (e.g., gene expression of antioxidant enzymes) to validate the findings. The assumption that *Sida acuta*'s benefits are solely antioxidant-driven may oversimplify its mechanism, warranting further exploration of anti-inflammatory or cytoprotective pathways.

The implications of this study are significant, particularly given the current date, July 04, 2025, and the ongoing global interest in natural remedies amid rising non-steroidal anti-inflammatory drug (NSAID)-induced complications. *Sida acuta* emerges as a promising candidate for adjunctive therapy, potentially reducing the therapeutic burden of synthetic drugs. Future research should focus on elucidating the active compounds, optimizing dosage regimens, and conducting clinical trials to translate these findings to human

applications. Additionally, long-term studies are recommended to assess safety and efficacy, addressing the current gap in chronic exposure data.

In summary, *Sida acuta* aqueous extract demonstrates a potent ability to protect liver function and prevent gastric ulceration in indomethacin-treated rats, offering a natural alternative with broad therapeutic potential. This study contributes to the growing body of evidence supporting phytotherapy, challenging the pharmaceutical-centric narrative, and lays the groundwork for further scientific inquiry into its clinical utility.

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