COMPARATIVE STUDY OF THE GASTRO-PROTECTIVE POTENTIAL OF AQUEOUS EXTRACT OF *PENNISETUM PEDICELLATUM* ALONE AND IN COMBINATION WITH *SORGHUM BICOLOR* IN RATS.

BY

EMAKHU OSEAHUME SAMUEL

PHA1404249

DEPARTMENT OF PHARMACOLOGY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF DOCTOR OF PHARMACY (PHARMD) DEGREE

DEPARTMENT OF PHARMACOLOGY

FACULTY OF PHARMACY

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JANUARY, 2023.

CERTIFICATION

This is to certify that this project work was carried out by EMAKHU OSEAHUME SAMUEL in the Department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin City.

……..

DR. F. C. AMAECHINA **Signature and Date**

(HEAD OF DEPARTMENT)

……

PROF. S.O. OKPO **Signature and Date**

(SUPERVISOR)

……

EMAKHU OSEAHUME SAMUEL **Signature and Date**

(STUDENT)

DEDICATION

This work is dedicated to God Almighty the source of my life and the very essence of my being.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to the following individuals and entities for their invaluable support and contribution to my final year project:

First and foremost, I would like to extend my heartfelt thanks to my supervisor, Prof. S. O. Okpo, for his invaluable guidance, support, and patience throughout the project. His expertise and insights have been invaluable, and I am deeply grateful for his mentorship.

I would like to thank my parents for their unwavering love, support, and encouragement throughout my academic journey. Their guidance and sacrifice have been invaluable, and I am deeply grateful for all that they have done for me.

I am also grateful to my uncles, Roberts Esheimokhai, Ehime Urieto and Kehinde Sotonwa, who has been a constant source of inspiration and motivation. His belief in me and his wise counsel have helped me to overcome many challenges and stay focused on my goals.

I am also grateful to the Head of Department, Dr. Fabian Amaechina, for his support and encouragement throughout my studies. His leadership and guidance have been instrumental in my academic and professional development.

I would also like to thank my coursemates, Cyril Eju, Tobias Odoh, Unegbu Nelson, Jude Aisowan, Justice Onile, Emmanuel Odiegbe, Habeeb Tijani, Ezomo Efosa, Moses Amadin, Elusakin Cyril, Ekene Jude, Maryanne Enawore and Abigail Aigbekan. My friends, Grace, Dapo, Kelly, Idris, Chibuike, Ojay and my siblings, Covenant and Sharon for their unwavering support and love throughout my journey.

Finally, I would like to thank the almighty God for His love, grace, and protection throughout this journey. Without His guidance and blessings, this project would not have been possible.

I am deeply grateful to all of the above for their invaluable support and contribution to my final year project. I could not have done it without them. Thank you."

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ABBREVIATIONS

PPAE - Aqueous extract of *Pennisetum pedicellatum*

PPSB - Aqueous extract of *Pennisetum pedicellatum* and *Sorghum bicolor*

ANOVA - One-way analysis of variance

ABSTRACT

Peptic ulcer disease is a set of conditions characterized by ulceration in the upper gastrointestinal tract. *Pennisetum pedicellatum* have historically been used to cure wounds, pain-related conditions, to feed animals, and to treat parasite diseases and eye infections.

The purpose of this study is to evaluate if *Pennisetum pedicellatum* alone or in combination with *Sorghum bicolor* aqueous extract has gastro-protective properties using chemically-induced (ethanol and indomethacin) ulcer model in rats. The reference treatment was ranitidine (100 mg/kg). All extracts and drugs were administered through the oral route. The results were analyzed using one-way analysis of variance(ANOVA) followed by Dunnet post hoc test.

The extract of *Pennisetum pedicellatum* (100 -500 mg/kg) significantly ($p<0.0001$) protected against ethanol- and indomethacin-induced ulcerations, with the greatest effect at 100 mg/kg. The extract also significantly reduced the severity of lesions. The aqueous extract of *Pennisetum pedicellatum* alone or in combination with *Sorghum bicolor* possesses anti-ulcer properties that may be due to cytoprotective mechanism or neutralization of gastric acid. These findings indicate that P*ennisetum pedicellatum* could have a beneficial role against ulcers.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Peptic ulcer disease (PUD) is a break in the inner lining of the stomach, the first part of the small intestine, or sometimes the lower esophagus. An ulcer in the stomach is called a gastric ulcer, while one in the first part of the intestines is a duodenal ulcer (Najm, 2011). Peptic ulcer disease is gradually becoming a public health emergency in contemporary society today with several predisposing factors such as high incidence of NSAIDs abuse as well as the discovery of several resistant strains of *Helicobacter pylori* bacteria. In recent times peptic ulcer disease has become a serious ailment that calls for urgent concern. Increase in use of NSAIDs as well as other unhealthy lifestyle choices have all contributed to the development of the is condition.

The history probably starts with identification of duodenal perforation by To Cheng in 1984 when he found a duodenal perforation in a preserved body of 167 BC in China (Lau and Leow*, 1997*). The discovery of this duodenal perforation led to however several theories about what the condition might suggest. The discovery of gastric ulcer will not come until years later in about 1670 in England.

In 1670 King Charles I's daughter, Henriette-Anne, died suddenly at the age of 26, a day after she complained of abdominal pain and tenderness. As poisoning was suspected an autopsy was performed that revealed peritonitis and a small hole in the anterior wall of the stomach (Bertleff and Lange, 2010). *Helicobacter pylori* was identified in 1982 by two Australian scientists, Robin Warren and Barry J. Marshall, as a causative factor for ulcers (Warren and Marshall, 1983). In 2005, the Karolinska Institute in Stockholm awarded the Nobel Prize in

Physiology or Medicine to Marshall and his long-time collaborator Dr. Warren "for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease". Marshall continues research related to *Helicobacter pylori* and runs a molecular biology laboratory at UWA in Perth, Western Australia. Development of therapies for gastric ulcer has begun to take a new turn as we have observed that several therapies involving the use of proton pump inhibitors and H_2 receptor antagonists are now being complemented with antibiotics for eradication of *Helicobacter pylori* infection as well as gastroprotective medications to aid in secretion of gastric mucus and encourage production of gastroprotective factors in the gastrointestinal tracts. There is currently a shift to the use of probiotics such as *Lactobacillus spp*.

1.2 Etiology of peptic ulcer disease

Peptic ulcer disease (PUD) has various causes; however, Helicobacter pylori-associated PUD and NSAID-associated PUD account for the majority of the disease etiology (Narayanan *et al., 2018).*

Common causes of ulcer includes: *Helicobacter pylori* infection, NSAIDs, Medications.

Not too common causes of gastric ulcer includes Zollinger-Ellison syndrome, Malignancy (gastric/lung cancer, lymphomas), Stress (Acute illness, burns, head injury), Viral infection, Vascular insufficiency, Radiation therapy, Crohns disease, Chemotherapy, Helicobacter Pylori-Associated PUD.

Helicobacter pylori is a gram-negative bacillus that is found within the gastric epithelial cells in the gastrointestinal tract. This bacteria is majorly responsible for 90% of duodenal ulcers and 70% to 90% of gastric ulcers commonly encountered. *Helicobacter pylori* infection is more prevalent among those with lower socioeconomic status and is commonly acquired

during childhood. The organism has a very wide spectrum of virulence factors allowing it to adhere to and inflame the gastric mucosa. This results in hypochlorhydria or achlorhydria, leading to gastric ulceration.

Virulence Factors of *Helicobacter Pylori*

Urease: The secretion of urease breaks down urea into ammonia and protects the organism by neutralizing the acidic gastric environment.

Toxins: CagA/VacA is associated with stomach mucosal inflammation and host tissue damage.

Flagella: Provides motility and allows movement toward the gastric epithelium.

NSAID-associated PUD

Nonsteroidal anti-inflammatory drugs use is the second most common cause of PUD after *H. pylori* infection. (Lanas *et al.,* 2015, Huang *et al.,* 2022). The secretion of prostaglandin normally protects the gastric mucosa. NSAIDs block prostaglandin synthesis by inhibiting the COX-1 enzyme, resulting in decreased gastric mucus and bicarbonate production and a decrease in mucosal blood flow.

1.3 Epidemiology

Peptic ulcer disease (PUD) is a global problem with a lifetime risk of development ranging from 5% to 10%. (Snowden, 2008; Lanas, 2017) with a ratio of 0.1% to 0.3% per year (Lanas and Chan, 2017). Disability-adjusted life year for peptic ulcer disease per 100000 inhabitants was over 220 patients in the test population (WHO, 2009). Peptic ulcer resulted in 301,000 deaths in 2013 down from 372,000 in 1990 (GBD, 2015).

In Western countries, the percentage of people with *H. pylori* infections roughly matches age (i.e., 20% at age 20, 30% at age 30, 80% at age 80, etc.). Prevalence is higher in third world countries, where it is estimated at 70% of the population, whereas developed countries show a maximum of a 40% ratio. Overall, *H. pylori* infections show a worldwide decrease, more so in developed countries. Transmission occurs via food, contaminated groundwater, or human saliva such as from kissing or sharing food utensils (Brown, 2000).

Peptic ulcer disease had a tremendous effect on morbidity and mortality until the last decades of the 20th century when epidemiological trends started to point to an impressive fall in its incidence. The reason that the rates of peptic ulcer disease decreased is thought to be the development of new effective medication and acid suppressants and the rational use of non steroidal anti-inflammatory drugs (NSAIDs) (Lanas and Chan, 2017).

Overall, there is a decrease in the incidence of PUD worldwide due to improved hygienic and sanitary conditions combined with effective treatment and judicious use of NSAIDs (Lanas, 2017). Duodenal ulcers are four times more common than gastric ulcers. Also, duodenal ulcers are more common in men than in the woman

1.4 Sign and Symptoms of peptic ulcer disease

Signs and symptoms of peptic ulcer disease may vary depending upon the location of the disease and age. Gastric and duodenal ulcers can be differentiated from the timing of their symptoms in relation to meals. Nocturnal pain is common with duodenal ulcers. Those with gastric outlet obstruction commonly report a history of abdomen bloating and or fullness.

Common signs and symptoms include: Epigastric abdominal pain radiating towards the thoracic region, bloating, abdominal fullness, nausea and vomiting, weight loss/weight gain, hematemesis, melena in severe cases.

Warning symptoms or alarm symptoms that should prompt urgent referral (Banerjee *et al*., 2010) include:

a. Unintentional weight loss,
b. Progressive dysphagia,
c. Overt gastrointestinal bleeding,
d. Iron deficiency anemia,
e. Recurrent emesis,
f. Family history of upper gastrointestinal malignancy.

1.5 Pathophysiology of peptic ulcer disease

The peptic ulcer disease (PUD) mechanism results from an imbalance between gastric mucosal protective and destructive factors. The protective factors includes mucus secretion, bicarbonate ion secretion to mop out excess hydrogen ions produced by gastric acid from the parietal cells, regulation of gastric blood flow all these work in synergy to maintain the integrity of the gastric mucosa.

Some of the risk factors predisposing to the development of PUD includes *H. pylori* infection, NSAID use First-degree relative with PUD, emigrant from a developed nation, African American/Hispanic ethnicity. All the above mentioned contribute in one way or the other to loss or depletion of the defensive mechanisms that have been put in place by the body to checkmate excess acid secretion which may lead to gastric ulcer. With peptic ulcers, there is usually a defect in the mucosa that extends to the muscularis mucosa. Once the protective superficial mucosal layer is damaged, the inner layers are susceptible to acidity. Further, the ability of the mucosal cells to secrete bicarbonate is compromised (Tan *et al*., 2012).

H.pylori is known to colonize the gastric mucosa and causes inflammation. The *H. pylori* also impairs the secretion of bicarbonate, promoting the development of acidity and gastric metaplasia. The presence of *H. pylori* is gradually becoming a menace in the pathophysiology of ulcer owing to rising development of very resistant strains to conventional antibiotics being used in therapy. Infection of abdominal sores by *H.pylori* contributes to an unending charade of symptoms of epigastric pain due to non-healing of the gastric sores (Shiotani and Graham, 2002).

The discovery of NSAIDs for the management of pains has led to a sudden rise in their use and abuse. NSAIDs work by inhibiting the enzyme, cylooxygenase, which is responsible for the synthesis of prostaglandins which are gastroprotective components of the gastrointestinal tract. NSAIDs has also been linked to gastrointestinal upsets, gastrointestinal bleeding and melena. In very severe cases, this culminates an array of risk factors responsible for gastric ulceration (Atchison *et al*., 2013).

Alcohol intake also causes eroding of the gastric mucus and thereby tampering with the protective mechanisms. Methylxanthines such as caffeine are also implicated in causing gastric ulceration; they inhibit the phosphodiesterase enzyme which is associated with inhibition of acid secretion in the gastrointestinal tract (Dziadus-Sokolowska *et al*., 1989).

1.6 COMPLICATIONS OF PEPTIC ULCER DISEASE (PUD)

Peptic ulcer of any aetiology can result in complications. PUD has four main complications: bleeding, penetration, perforation, and obstruction. The likelihood of complications may be increased in patients with giant ulcers and pyloric channel ulcers. The prevalence of PUD has grown recently, especially in the elderly (Hermansson *et al*., 1997; Bertleff and Lang, 2010).

1.7 Classification of peptic ulcer disease

Generally, peptic ulcers are divided into duodenal and gastric ulcer. There are major distinguishing features. A gastric ulcer is a sore or injury on the lining of the stomach, small intestine or the oesophagus. A duodenal ulcer is a peptic ulcer that develops in the first part of small intestine (duodenum).

Table 1: Differences between Gastric and Duodenal Ulcer

Gastric Ulcer	**Duodenal Ulcer**
Occurs in the stomach	Occurs in the duodenum
Epigastric pain occurs 1-2 hours after eating	Epigastric pain occurs 2-5 hours after eating
Heart burn, chest discomfort and early satiety are common symptoms experienced	Heart burn, chest discomfort are less common but may be seen
Pain less likely to wake up patients at night	Pain may awaken patient at night time leading to sleep discomfort
Abdominal pain cannot be relieved by eating	Abdominal pain can be relieved by eating
Hematemesis more common	Melena more common
Equal in both sexes	Occurs majorly in men
Weight loss	Weight gain
Pain before food intake	Pain after food intake
Aggravated by hunger	Aggravated by eating

While the differences highlighted above show a clear distinction between both types of gastric ulcers, the treatment plan and regimens used are largely similar which requires use of anti-secretory agents as well as antibiotics.

1.8 Diagnosis of Ulcer

Diagnosis of PUD requires proper patient history taking and evaluation, physical examinations of the abdominal region, and invasive/non-invasive medical techniques. Patient reporting of constant epigastric abdominal pain, early satiety, and fullness following intake of food may raise suspicion of PUD. The pain of gastric ulcers is usually felt 2 to 3 hours after a meal and may result in weight loss, whereas the pain of duodenal ulcers decreases with a meal which can result in weight gain this is a major distinguishing factor between the two types of peptic ulcer disease. Any patient presenting with symptoms such as anemia, melena, hematemesis, or a gradual loss of body weight should be further investigated for complications of PUD, predominantly bleeding, perforation, or cancer. A physical exam may reveal epigastric abdominal tenderness and signs of anemia. Gastric ulcers are most commonly located on the lesser curvature, whereas duodenal ulcers are most common at the duodenal bulb. The ulcer is round to oval with a smooth base. Acute ulcers have regular borders, while chronic ulcers have elevated borders with inflammation. An ulcer extends beyond the muscularis mucosa. Several diagnostic techniques have been accepted over the years for the diagnosis of peptic ulcer disease and these techniques includes-

1. Esophagogastroduodenoscopy (EGD): This is the gold standard and most accurate diagnostic procedure with a high range of sensitivity and specificity up to 90% in detecting the presence of gastric and duodenal ulcers. The American Society of Gastrointestinal Endoscopy has published guidelines on the role of endoscopy in patients presenting with upper abdominal pain or dyspeptic symptoms suggestive of PUD (Banerjee *et al*., 2010) Patients over 50 years of age and new onset of dyspeptic symptoms should get evaluated by an EGD. Anyone with the presence of alarm symptoms should undergo EGD irrespective of age.

2. Barium swallow: It is also another reliable diagnostic tool, indicated when EGD is contraindicated.

3. Complete blood work: which includes liver function test, and levels of amylases and lipases.

4. Serum gastric acid level determination: Serum gastric acid level is ordered if Zollinger Ellison syndrome is suspected. Zollinger Ellison syndrome is a disease condition that is characterized by excess secretion of gastric acid due to the presence of parietal cell tumors.

5. *Helicobacter pylori* testing: Urea breath test is common diagnostic tool used in the detection of *Helicobacter pylori* infection in the gastric mucosa. It is one of the most common methods used and it is cheap and widely available. Urea breath test has a high sensitivity and specificity. It may be used to confirm eradication after 4 to 6 weeks of stopping treatment. In the presence of urease, an enzyme produced by *H.pylori*, the radio-labeled carbon dioxide produced by the stomach is exhaled by the lungs.

6. Serologic testing: Antibodies to *H.pylori* can also be measured and detected.

7. Urine-based ELISA and rapid urine test

8. Endoscopic biopsy: Culture is not generally recommended as it is expensive, time-consuming, and invasive. It is indicated if eradication treatment fails or there is suspicion about antibiotic resistance. Biopsies from at least 4-6 sites are necessary to increase sensitivity. Gastric ulcers are commonly located on the lesser curvature between the antrum and fundus. The majority of duodenal ulcers are located in the first part of the duodenum.

9. Computerized tomography: Computerized tomography of the abdomen with contrast is of limited value in the diagnosis of PUD itself but is helpful in the diagnosis of its complications like perforation and gastric outlet obstruction.

1.9 ANIMAL MODEL OF PEPTIC ULCER DISEASE

Anti-ulcer medications are assessed using a variety of models. The selection of an appropriate model, however, has proven to be challenging because each model has both major benefits and drawbacks. Local resources, study goals, the hypothesis being tested, or research problems being addressed by the researcher can all have an impact on the model that is chosen. The model of choice may also be influenced by its applicability to the particular type of peptic ulcer illness being studied. To examine the mechanisms of action of drug candidates with anti-ulcer efficacy, pre-clinical investigations should typically be conducted in vivo and complemented, when possible, with in vitro research.

In various animal species, physiological, pharmacological, or surgical interventions can cause peptic ulcers. However, rats are used in the majority of peptic ulcer research investigations. The following models are used experimentally to test or assess a substance's anti-peptic ulcer activity:

a) water-immersion stress or cold-water-restraint or cold-restraint stress,
b) NSAIDs- (indomethacin, aspirin, and ibuprofen) induced gastric ulcers,
c) ethanol-induced gastric ulcers,
d) acetic acid-induced gastric ulcers,
e) histamine-induced gastric ulcers,
f) serotonin-induced gastric ulcers,
g) pylorus-ligated-induced peptic ulcers.

1.9.1 Water-Immersion-Stress-Induced Ulcer Model

People who experience many types of psychological and physical stress can develop stomach ulcers (Demirbilek, 2004), and rat models have been created to replicate the human disease

state. This model uses the Brodie and Hanson (1960) constraint technique along with the immersion approach in either cold water or regular water by Levine (1971). According to reports, using both of these techniques together effectively causes acute stress lesions in rats (Senay and Levine, 1967), arising mainly from physiological discomfort. It is known that both morphologically and histopathologically, gastric ulcers caused by cold-water-restraint stress (CWRS), cold-restraint stress (CRS), or water-immersion stress (WIS) in rats or mice mimic human peptic ulcers (Konturek *et al*., 2003). This commonly used model is reportedly helpful for determining or examining the effects of medications on the recovery of ulcers in rats.

The pathophysiology of ulcers brought on by stress is complicated. Histamine is released, which causes an increase in acid secretion, a decrease in mucus production (Kitagawa *et al*., 1979), pancreatic juice reflux, and a poor flow of gastric blood (Guth, 1972), all of which contribute to the development of ulcers. Stress also increases gastrointestinal motility, which results in folds in the stomach (Peters and Richardson, 1983) that are more vulnerable to harm when they come into touch with acid (Brodie and Hanson, 1960). Additionally, it has been discovered that stress lowers the quantity and quality of mucus that adheres to the gastrointestinal mucosa.

1.9.2 Non-Steroidal Anti-inflammatory Drugs (NSAIDs) Induced Mucosal Damage

Gastric ulcers are a known side effect of non-steroidal anti-inflammatory drugs (NSAIDs), particularly when misused. Examples of NSAIDs include indomethacin, aspirin, and ibuprofen. The development of NSAID-induced gastric ulcer models in rats has made use of this phenomena. Since mucosal prostaglandin synthesis and gastric acid secretion are involved in the pathogenesis, the model is crucial for examining the potential usefulness of anti-secretory and cytoprotective drugs. It is the ulcer model used in anti-ulcer investigations

the most commonly. The prevalence of use may be explained by the fact that *Helicobaceter pylori* and NSAID-induced peptic ulcers are the two most frequent causes of peptic ulcers, respectively.

By blocking prostaglandin synthetase in the cyclooxygenase pathway, NSAIDs are known to cause ulcers. The two drugs that are most frequently used to induce ulcers are aspirin and indomethacin.

1.9.3 Ethanol-Induced Peptic Ulcer Model

Alcohol consumption is thought to increase the risk of stomach ulcers. It easily reaches the stomach mucosa owing to its capacity to dissolve the protective mucous and expose the mucosa to the proteolytic and hydrolytic effects of hydrochloric acid and pepsin (Oates and Hakkinen, 1988), resulting in damage to the membrane (Sener *et al*., 2004). Additionally, alcohol disrupts the vascular endothelium and increases vascular permeability, which causes acid secretion to increase and blood flow to decrease, resulting in microvascular damage. Also, it makes xanthine oxidase more active. By having a direct toxic effect, ethanol also causes necrotic lesions in the gastric mucosa of animals, which decreases the release of bicarbonates and decreases the amount of gastric mucus that animals generate (Marhuenda *et al.*, 1993).

The development of the ethanol model of peptic ulcers took advantage of ethanol's harmful effects. The model resembles acute peptic ulcers in humans and is independent of gastric acid output (Brzozowsk *et al*., 1998). Due to the lack of gastric acid secretion, which is necessary for the formation of the ulcer, the ethanol-induced ulcer model may not be suitable or useful for evaluating the efficacy of antisecretory medications or testing materials. Instead, the ethanol-induced ulcer model is helpful for evaluating the effectiveness of possible medications or testing substances with antioxidant and/or cytoprotective properties.

1.9.4 Acetic Acid-Induced Gastric Ulcers

The chronic nature of peptic ulcer, which is defined by recurrent phases of healing and re-exacerbation, is one of the least understood elements of the disease. This presents a challenge to both patients and doctors. Most experimental ulcerative lesions do not spontaneously re-ulcerate, and they heal within a few days without leaving a scar. By injecting acetic acid submucosally into rats, (Takagi et al., 1970) created a model for creating chronic stomach ulcers, and they documented the healing of the lesions over long periods of time following the ulcer production. Due to the experimental gastric ulcer's long-term durability, histological and gross similarity to a chronic ulcer in a human, it was classified as chronic.

The acetic acid model is appropriate for peptic ulcers that are persistent. It is used to assess how potential medications or testing materials affect the progression of chronic peptic ulcer healing as well as to check for their antisecretory and cytoprotective effects (Takagi et al., 1970; Okabe et al., 1971). In the stomach and duodenum of mice, rats, Mongolian gerbils, guinea pigs, cats, dogs, miniature pigs, and monkeys, the model reliably and readily causes round, deep ulcers (Okabe and Amagase, 2005).

1.9.5 Histamine-Induced Gastric Ulcer

The histamine model for ulcer production is based on the notion that several factors, including the release of histamine, mediate gastric ulceration. In addition to increasing the formation of stomach acid, histamine also disrupts the gastric mucosa, microcirculation, aberrant motility, and mucus production. Histamine causes gastric ulcers by powerfully boosting the stomach's acid production and dilation of the blood vessels, which increases vascular permeability (Cho and Pfeiffer, 1981; Watt, 1959). The histamine-induced ulcer

model is based on these pharmacological properties of histamine, which also contribute to the model's usefulness in assessing the antisecretory effects of possible ulcer-treating medications and H2-receptor antagonists.

1.9.6 Serotonin-Induced Gastric Ulcer

Serotonin has been used to produce ulcers. It is understood to induce vasoconstriction, which lowers gastric mucosal blood flow (GMBF) and results in an acute mucosal damage (LePard and Stephens, 1994).

1.9.7 Pylorus-Ligated-Induced Peptic Ulcer (Shay's Method)

Pylorus ligation causes ulcers, which can be used as a model to study how well medications affect gastric secretions. Stomach ulcers are brought on by the buildup of gastric acid following pyloric end ligation. These ulcers are brought on by the gastric mucosa's auto-digestion, which causes the gastric mucosal barrier to break down. In other words, mucosal digestion may result from an increase in acid-pepsin buildup caused by pylorus constriction. The model is helpful for assessing how anti-secretory medications, which lessen the secretion of gastric aggressive factors like acid and pepsin, affect the body. The model is helpful for evaluating the cytoprotective properties of medications that stimulate mucus secretion.

1.10 DRUG TREATMENT OF PEPTIC ULCER DISEASE (PUD)

Regulating the aggressive components has been the main focus of pharmacologic treatment for ulcer disease. Investigations have, however, also concentrated on substances that could strengthen the protective properties. The pharmacologic treatments that are currently in use and under development can be divided into three groups:

a) Acid neutralizing agent,

b) Antisecretory agents, and

c) Agents that improve mucosal defense.

Theoretically, antacids work through a variety of mechanisms to encourage ulcer cure. They are weak hydrochloric acid buffers first and foremost. This lessens the acid load, cuts down on the amount of hydrogen ions available for back-diffusion, and prevents pepsinogen from being converted to pepsin. Second, bile salts, pepsin, and lysolecithin, which are regarded as erosive agents, may be bound by antacids. Last but not least, it is hypothesized that antacids have cytoprotective properties connected to prostaglandin production.

Deleted[Zangote]: **1.10.1 ACID NEUTRALIZING AGENTS**

The majority of antacids are made of magnesium hydroxide, aluminum hydroxide, or calcium carbonate and are available in a range of dosage forms, formulations, and acid-neutralizing characteristics (Berardi, 1989).

1.10.2 ANTISECRETORY AGENTS

The main pharmacologic mechanism of action of the medications that can be categorized as antisecretory medicines involves interfering with the secretion of stomach acid. The parietal cells that border the gastric mucosa have been found to include receptors for histamine, acetylcholine, and gastrin, three chemical mediators of acid secretion (Bertaccini and Coruzzi 1985). The different class of antisecretory agents are discussed below;

Histamine$_2$ (H_2)-receptor antagonist: Cimetidine was the first histamine2-receptor antagonist made commercially in the United States. Currently, there is ranitidine, famotidine, and nizatidine. By competitively inhibiting the H2-receptors found on the parietal cells, the H2-receptor antagonists reduce stomach acid output. These substances have no discernible effects on sympathetic, muscarinic, nicotinic, or histamine receptors. The volume and concentration of hydrogen ions in gastric contents are decreased by this reversible and dose-dependent reduction of gastric-acid production in both the baseline and nocturnal states as well as after

stimulation by histamine, pentagastrin, insulin, food, the vagal reflex, and caffeine (Henn *et al.*, 1975; Longstreth *et al*., 1976).

ANTICHOLINERGIC (ANTIMUSCARINIC) AGENTS:

By inhibiting the acetylcholine receptors on the parietal cells, the anticholinergic drugs decrease the production of stomach acid. Atropine, a tertiary amine molecule, and propantheline, a quaternary ammonium compound, are members of this category of drugs. Very little information about the reported effectiveness of anticholinergic drugs in treating ulcer patients is available from well-controlled research. Due to the fact that these medications do not significantly reduce acid secretion compared to H2-receptor antagonists, they are not suggested for the treatment of peptic ulcer disease. Patients furthermore encounter anticholinergic side effects include dry mouth, impaired vision, constipation, urine retention, tachycardia, and delayed stomach emptying when the medications are used at therapeutic levels (Berardi, 1989).

PROTON PUMP INHIBITORS

These medicines belong to a group that significantly and persistently reduces the production of stomach acid. They accomplish this by permanently blocking the H+/K+ ATPase proton pump in the stomach. PPIs are the most effective acid suppressants currently on the market and perform noticeably better than histamine H2 receptor antagonists. Examples in this class includes Omeprazole, Esomeprazole, Rabeprazole, Pantaprazole.

1.10.3 AGENTS THAT IMPROVE MUCOSAL DEFENSE

The release of mucus and bicarbonate, the stomach mucosa's microcirculation, the rapid turnover of epithelial cells, and cytoprotection from endogenous substances are a few of the gastric mucosa's defensive mechanisms. Numerous studies have looked into the use of

pharmacologic intervention to treat or prevent ulcers via one of the aforementioned mechanisms.

SUCRALFATE

Sulfated sucrose and aluminum hydroxide combine to form a complex of this aluminum salt of a sulfated disaccharide. Sucralfate is not an antacid. A protective protein barrier that is adherent and formed at the location of the mucosal lesion is produced when it combines with protein (McGraw and Caldwell, 1981).

PROSTAGLANDINS

Research and development of prostaglandin analogs as prospective treatments for people with peptic-ulcer illness have been sparked by the realization that endogenous prostaglandins have cytoprotective effects. A prostaglandin E1 analog called misoprostol has been authorized for the treatment of ulcers brought on by non steroidal anti-inflammatory drugs. These synthetic analogs work well when given orally to prevent ulcers brought on by alcohol and drugs, to prevent ulcers brought on by stress, and perhaps even to heal stomach and duodenal ulcers (Nicholson, 1985).

The primary goal of treatment for *H pylori*-associated ulcer illness is to eradicate the infection. Antibiotics and acid-inhibiting treatment are typically used to accomplish eradication. Healing can occur with just antibacterial therapy, but adding acid suppressants (ie, PPIs) speeds up the process (Hosking *et al*., 1994). The triple therapy of a PPI twice daily, clarithromycin 500 mg twice daily, and either amoxicillin 1 g twice daily (PPI-CA) or metronidazole 500 mg twice daily (PPI-CM) for 7–14 days is the recommended first-line treatment (Malfertheiner *et al*., 2002). The choice of antibiotics used depends on factors such as poor adherence and bacterial resistance that might cause treatment failure. Because

bacterial resistance to metronidazole is higher than bacterial resistance to amoxicillin, amoxicillin is preferred over metronidazole in first-line therapies (Lahaie and Gaudreau, 2000). As a first-line treatment, quadruple therapy, which consists of bismuth 120 mg four times daily, metronidazole 500 mg three times daily, tetracycline 500 mg four times daily, and a PPI twice daily for at least seven days, has also gained recognition (Laine, 2003). If a first-line treatment fails, second-line therapy is recommended, but it shouldn't contain clarithromycin or metronidazole (Chey *et al.*, 2017). PPI, amoxicillin, and levofloxacin triple therapy for 14 days appears to be an effective treatment, achieving eradication rates between 74 to 81%. (Chen *et al*., 2016).

1.11 HERBAL MEDICINE IN THE MANAGEMENT OF PEPTIC ULCER DISEASE (PUD)

Phytotherapy, the practice of using medicinal plants to treat a variety of illnesses, is as old as humankind. Additionally, in recent years there has been an increase in interest in alternative therapies and the use of herbal medicines, particularly those made from medicinal plants (Yesilada *et al*., 1999). Additionally, medicinal plants are regarded as the main source of potentially new medications due to the occurrence of diverse adverse effects from the use of conventional therapies for a variety of disorders. The most important sources of novel medications are plant extracts and their compounds, which have also been demonstrated to have promising outcomes in the treatment of gastric ulcers (Falcão *et al*., 2008).

The therapeutic capabilities of medicinal plants are a result of their ability to create a wide range of secondary metabolites, or phytochemical components, which are renewable and diverse. Many plants have therefore exploited these phytochemicals as a defense mechanism against diseases (Abdallah, 2011). Below, some herbal medications with antiulcer efficacy are discussed, paying particular attention to their mechanisms of action.

Azadirachta indica A.

It is a member of the Meliaceae family and is frequently referred to as Neem. It has been found to have strong antiulcer and gastroprotective properties. Preventing acidpepsin secretion is the main mechanism. Neem does not affect mucin secretion, but it does lengthen the life of mucosal cells, as indicated by a reduction in cell shedding in the stomach acid. Therefore, rather than on the secretion of defensive mucin, its anti-secretory and proton pump inhibitory function is responsible for the ulcer preventive activity. By inhibiting lipid peroxidation and scavenging the endogenous hydroxyl radical (OH), the primary cause of ulcers, it prevents oxidative damage to the gastric mucosa (Dorababu *et al*., 2006; Maity *et al*., 2009).

Momordica charantia L.

It is a member of the Cucurbitaceae family and is also known as bitter gourd and karela. methanolic extract of Momordica charantia fruit treats gastric and duodenal ulcers. The enhanced mucus secretion and anti-stress properties of its component were hypothesized to be responsible for the antiulcer efficacy. Peptic ulcers did respond favorably to the olive oil extract of the M. charantia fruit (Gurbuz *et al.*, 2000).

Panax ginseng (Korean red ginseng)

It is a specie of ginseng belonging to the family Araliaceae. They have healing effect on gastric ulcers. Ginseng species' alleged mechanisms are mucin secretion significantly increased and inhibited H+/K+ ATPase activity and malondialdehyde (MDA) in the abdomen (Oyagi *et al*., 2010).

Ocimum sanctum

It is a member of the Lamiacae family and is frequently referred to as Tulsi. In India, the Hindus revere it as a sacred plant. Advanced research on this plant has reportedly shown that it possesses antiulcer properties. Because of the fixed oil's lipoxygenase inhibitory, histamine-antagonistic, and anti-secretory properties, it has been demonstrated to have antiulcer activity (Singh and Majumdar, 1999; Vinod *et al.*, 2011).

Aloe barbadencis

Aloe vera is a common name for it. it belongs to the family a sphodelaceae (Lilliaceae). it possesses gastroprotective properties. The mucilage tissue at the center of leaves in this plant called aloe gel is used for a range of medicinal benefits. Its healing property is due to a compound referred to as glucomannan, which is enriched with polysaccharides like mannose. The glucomannan affects fibroblast growth factor and stimulates the activity and proliferation of these cells. The mucilage of aloe vera not only increases amount of collagen on wound site, but also increases transversal connections among these bands rather than changing collagen structure fastening wound healing (Keshavarzi *et al*., 2014).

1.12 THE PLANTS, *Pennisetum pedicellatum and Sorghum bicolor*

Pennisetum pedicellatum

P. *pedicellatum* is a many branched leafy annual grass up to 1m high. The culms are upright and branched, and the flat, glabrous leaves are 15–25 cm long by 4–10 mm wide. The inflorescence is a cylindrical, densely flowered, pink to purple panicle. The 4 mm long spikelets are typically single (Wipff, 2010). It is native to west africa and first introduced to india from where it has spread to south east asia and northern australia (Schmelzer, 1996).

Figure 1: Picture of *Pennisetum pedicellatum*

TAXONOMY

Kingdom; Plantae

Phylum; Tracheophyta

Class; Liliopsida

Order; Poales

Family; Poaceae

Genus; Pennisetum

Species; Pennisetum pedicellatum Trin.

Common name; Deenanth

CHEMICAL COMPOSITION

Phenols, tannins, alkaloids, saponins, anthraquinones and cardiac glycoside, steroids, anthocyanin phenols, terpenoids

USES

Pennisetum pedicellatum is an aggressive weed, used for treatment of wounds, pain related condition, feeding animals,eye and parasitic infections among the traditional healers in Kebbi State, Nigeria.

Sorghum bicolor

Sorghum (*Sorghum bicolor L.*) is a cereal of the family Poaceae, native to Africa, and was domesticated between 3,000 and 5,000 years ago (U.S. Grains Council, 2004). It is the fifth most produced cereal in the world, and is preceded by wheat, rice, maize, and barley (Food and Agricultural Organization, 2010). Around the world, there are over 7,000 varieties of sorghum (Kangama & Rumei, 2005). The sorghum grain has three distinct anatomical structures called the pericarp, endosperm, and germ. Some varieties have a fourth structure called the testa, located between the pericarp and the endosperm (Earp *et al*., 2004).

Figure 2: Picture of *Sorghum bicolor*

TAXONOMY

Kingdom; Plantae

Division; Magnoliophyta

Class; Liliopsida

Order; Poales

Family; Poaceae

Genus; Sorghum

Species; *Sorghum bicolor*

Common name; Guinea corn, Sorghum

CHEMICAL COMPOSITION

Amylose, amylopectin, proteins which are classified as prolamins and non prolamins, oleic acid, linoleic acid, linolenis acid, palmitic acid, phenolic acids, tannins, flavonoids, Stilbenes, Polycosanols and phytosterols

USES

It is currently unknown whether consuming chemicals extracted from sorghum, especially the whole grain, will have any functional advantages for human health. In vitro and animal studies have demonstrated that phenolics or fat-soluble chemicals extracted from sorghum have positive effects on the gut microbiota, where they may have effects similar to prebiotics (Cardona *et al*., 2013). Additionally, sorghum possesses resistant starch and dietary fiber, which is able to modify gut microbiota (Scott, *et al*., 2008; Martínez, *et al*., 2010).

The parameters associated with noncommunicable diseases such obesity, diabetes, dyslipidemia, cardiovascular disease, cancer, and hypertension can also be modulated by sorghum. According to studies, sorghum with a high tannin content prevents animals from gaining weight. s (Al-Mamary et al., 2001; Muriu, *et al*., 2002).

Recent research suggests that the phenolic chemicals in sorghum fractions influence how animals' glucose metabolism is regulated. Studies on animals have demonstrated that phenolic extracts of sorghum display a hypoglycemic effect comparable to glibenclamide, an anti-diabetic drug employed in the control group, because of its potent impact on plasma glucose and insulin (Chung *et al*., 2011).

The lipidic and phenolic fractions of sorghum affect variables associated with dyslipidemia and the risk of cardiovascular disease, according to in vitro and animal research. Phytosterols, polycosanols, and phenolic chemicals, which may affect cholesterol absorption, excretion, and synthesis, are responsible for these advantages. By modifying the gut bacteria, the lipid fraction may also change how much cholesterol is absorbed (Martínez, *et al*., 2009).

A recent finding in the scientific literature suggests that sorghum can lower blood pressure. Angiotensin I converting enzyme activity was reduced in this investigation by an isolate of sorghum, α-kafirins in both competitive and non-competitive ways (Kamath *et al*., 2007).

The prevalence of esophageal cancer among black South Africans increased once corn replaced sorghum as a major item in the diet (Isaacson, 2005). It is yet unclear how sorghum reduced people's risk of developing esophageal cancer.

1.13 RATIONALE FOR THE STUDY

Drugs made from herbs have been used extensively throughout history. Oral tradition has been passed down down the generations based on religious convictions and/or life experience.

When powerful chemicals were first isolated in the 19th century, the earliest attempts to offer scientific proof occurred. Since then, phytotherapy has been based on the contemporary pharmacology hypothesis. In order to propose an effective and safe dose for a pharmacological indication, studies have attempted to unravel the molecular mechanism of each molecule. Clinical studies conducted in stages have shown the therapeutic advantages of using herbal drugs, particularly for chronic disorders. Although herbal medicines have pleiotropic effects, there is still a need for a comprehensive, rational, and widely recognized theory that can account for the effectiveness of phytotherapy.

1.14 AIMS AND OBJECTIVE

The aim of this study is to investigate the in-vivo gastro-protective potential and comparative effect *Pennisetum pedicellatum* alone, and in combination with *Sorgum bicolor.*

The objectives of the study are to:

1. evaluate the phytochemical constituents of the extract of *Pennisetum pedicellatum* alone, and in combination with *Sorgum bicolor.*
2. determine the acute toxicity and LD_{50} dose of the extract of *Pennisetum pedicellatum* alone, and in combination with *Sorgum bicolor.*
3. evaluate the effect of *Pennisetum pedicellatum* alone, and in combination with *Sorgum bicolor* in peptic ulcer disease, using chemically-induced (ethanol and indomethacin) ulcer model in rats.

CHAPTER TWO

Deleted[Zangote]: ONE

MATERIALS AND METHODS

2.1 Plant material

The plant, P*ennisetum pedicellatum*, *and* inflorescence of *Sorghum bicolor* were obtained in the month of November from Gbaupe, Abuja Municipal Area council of FCT. It was identified by Dr Henry A. Akinnibosun of the Department of Plant biology and biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State.

2.2 Plant preparation

The aerial part of the plant P*ennisetum pedicellatum (PP)* and influorescence of *Sorghum bicolor (SB)* were washed, and chopped into smaller size.

Aqueous extract of *Pennisetum pedicellatum* (PPAE): Extraction was performed by weighing 400 g of the plant *Pennisetum pedicellatum (PP)* and was aseptically transferred into a 5000 ml round button flask, 4000 ml of distilled water was added and boiled at a temperature of 50°C for five (5) hours. The resultant mixture gotten was filtered using a funnel, cotton wool and a white cloth. The filtrate volume gotten was 2300 ml and was then concentrated using water bath and beaker. The extract was properly stored in the refrigerator.

Aqueous extract of *Pennisetum pedicellatum* and *Sorghum bicolor* (PPSB): Extraction was performed by weighing 400 g of the *Pennisetum pedicellatum* (PP) and 400 g of *Sorghum bicolor* (SB) and was aseptically transferred into a 5000 ml round button flask and 4000 ml of distilled water was added and boiled at a temperature of 50°C for five (5) hours. The resultant mixture gotten was filtered using a funnel, cotton wool and a white cloth. The filtrate volume gotten was 2000 ml and was then concentrated using water bath and beaker. The extract was properly stored in the refrigerator.

2.3 Animals

Male wistar rats (185 ± 45g) and Swiss albino mice (26 ± 7g) were obtained from the Laboratory Animal Centre of University of Benin, Benin City, Edo state. The animals were maintained under standard diet (Topfeeds Premier Mills Co. Ltd, Ibadan, Oyo state) and water *ad libitum*. All animals were acclimatized for two weeks and fasted overnight, with free access to water prior, to experiments. Animals were handled according to the protocol outlined in the "Principles of Laboratory Animal Care" National Institute of Health Guide for Care and Use of Laboratory Animals, Pub No. 85 - 23, revised 1985.

2.4 Drugs and reagents.

Absolute ethanol, Ranitidine (Laborate Pharmaceutical, India), Indomethacin (Yangzhou Norier Pharmaceutical, China), 2% Sodium carbonate, Formal Saline (10% neutral buffer formalin), Normal saline.

2.5 Phytochemical screening

Qualitative tests for the presence of plant secondary metabolites such as carbohydrate, reducing sugars, tannins, saponins, glycosides, and alkaloids were carried out on the extracts gotten (Sofowora, 1982; Trease and Evans 1985).

A 2 g quantity of the PPAE or PP/SB was dissolved in 20ml of distilled water. The filtrate was then used for the following tests:

Molisch test for carbohydrates

To 2 ml of the filtrate, 2 drops of 1% alcoholic α-naphtol were added followed by 2 ml of concentrated sulphuric acid inclined at an angle of 45°. A deep violet ring produced at the interphase between the liquids indicates the presence of carbohydrates.

Test for reducing sugars

To 2 ml of the filtrate, 2 drops of Benedict's reagent (mixture of equal volumes Fehling's solution A and B) were added and the mixture was then heated for 3 minutes. A green coloured solution or bricks red precipitate indicates the presence of reducing sugars.

Test for saponins

To 2 ml of the filtrate, 10 ml of distilled water was added. Expected observation for a positive result; Persistent frothing upon shaking showed the presence of saponins.

Test for tannins

To 2 ml of the filtrate, one drop of aqueous ferric chloride was added. A blank test was done by adding 2 drops of 5% ferric chloride solution to 5 ml of distilled water. A blue black coloration indicates the presence of tannins.

Test for alkaloids

To 2 g of the extract, 20ml of distilled water was added.

To 2 ml of the filtrate, two drops of Dragendorff's reagent was added. Expected observation for a positive result; formation of a reddish brown precipitate.

To 2 ml of the filtrate, 2 drops of Wagner's reagent was added. Expected observation for a positive result; formation of a brown precipitate.

To 2 ml of the filtrate, 2 drops of Hager's reagent was added . Expected observation for a positive result; formation of a yellow precipitate.

To 2 ml of the filtrate, 2 drops of Mayer's reagent was added. Expected observation for a positive result; formation of a milky precipitate.

Test for flavonoids

To 2 g of the extract, 20 ml of distilled water was added. The filtrate was used for the following tests:

Lead acetate test: To 5 ml of the filtrate, 10% lead acetate solution was added. Expected observation for a positive result; formation of a milky precipitate.

Sodium hydroxide test: To 5 ml of the filtrate, a few drops of 20% sodium hydroxide solution followed by a few drops of dilute hydrochloric acid solution was added. Expected observation for a positive result; formation of an intense yellow precipitate which dissolves on addition of dilute acid.

Ferric chloride test: To 2 ml of the filtrate, 8 ml of water was added. A few drops of 10% ferric chloride were later added. A green or blue solution indicated a positive result.

2.6 Pharmacological tests

2.6.1 Acute toxicity

Using the Lorke's method, which involves 2 phases:

In phase 1, Swiss albino mice were grouped into three(3) groups, each group containing three(3) mice. Stock solutions of the extracts PPAE and PPSB were prepared using strength of 10, 100 and 1000 mg/kg.

The extract PPAE and PPSB were administered through the oral route and they were observed for 24 hours.

In Phase 2, Swiss albino mice are grouped into 3 groups, each group contains one (1) mice. Using a stock solution of 1600, 2900 and 5000 mg/kg for each of the groups respectively.

The extract PPAE and PPSB were administered through the oral route and they were observed for 24 hours.

The mortality rate within a 24hour period was recorded. All animals were observed for a further 2 weeks for any latent signs of delayed toxicity.

2.6.2 Ethanol-induced ulcers

The rats were randomly divided into eight (8) groups of five(5) animals each and starved for 48 hours but had free access to water. Water was however withdrawn 2 hours before experiments.

Group 1 served as the control was treated with distilled water (5 ml/kg) orally. Groups 2, 3 and 4 was pre-treated with 100, 200 and 500 mg/kg of PPAE (aqueous extract of P*ennisetum pedicellatum*) respectively while Groups 5, 6 and 7 was pre-treated with 100, 200 and 500 mg/kg of PPSB (aqueous extract of P*ennisetum pedicellatum* and *Sorghum bicolor)* respectively and Group 8 received ranitidine (100 mg/kg) by oral intubation (Robert, 1979).

One hour later, 1 ml of absolute ethanol was administered by intragastric instillation to all the groups. One hour following ethanol administration, the animals were sacrificed one hour later by cervical dislocation and the stomach was isolated, opened along the greater curvature and washed.

Macroscopic examination of the stomachs of the animals in all the groups was done. The presence of ulcers was counted using a magnifying glass. The length of the ulcer was measured using a vernier caliper and scored on a scale of 0-10 (Martin *et al.,* 1988) as follows:

Grade or Score/ Description:

0= no ulcer

1= haemorrhagic and slightly dispersed ulcers, less than 2mm length

2= one ulcer as above, up to 2mm length.

3= more than one ulcer grade 2

4= one ulcer above 5mm length

5= more than one grade 4 ulcer

6= one ulcer up to 8mm

7= more than one ulcer grade 6

8= one ulcer above 8mm length

9= more than one grade 8 ulcer

10 = total ulceration and haemorrhage

The ulcer index (UI) was calculated thus:

$UI = U_N + U_S + U_P \times 10^{-1}$

Where: Un = Average number of ulcers per animal

Us = Average of severity score

Up = Percentage of animals with ulcers

2.6.3 Indomethacin-induced ulcers

The rats randomly divided into eight (8) groups of five(5) animals each and starved for 24 hours but had free access to water. Water was however withdrawn 2 hours prior to the experiment.

Group 1 which served as the control was administered with distilled water (5 ml/kg) orally. Groups 2, 3 and 4 were pre-treated with 100, 200 and 500 mg/kg of PPAE respectively while Groups 5, 6 and 7 was pre-treated with 100, 200 and 500 mg/kg of PP/SB respectively and Group 8 received ranitidine (100 mg/kg) by oral intubation, one hour before oral administration of indomethacin (40 mg/kg in 2% sodium carbonate solution) (Robert, 1979, Franzone *et al*.,1988). Six hours later, each rat was sacrificed by cervical dislocation and the stomach removed.

Formal saline (10% neutral buffer formalin) was injected into the totally ligated stomach for overnight storage. The next day, the stomach was opened along the greater curvature and washed in Normal saline.

Macroscopic examination of the stomachs of the animals in all the groups was done. The presence of ulcers was noted and scoring of the ulceration was done according to the method of Martin *et al*., (1988).

2.7 Statistical Analysis

Data were expressed as the mean ± standard error of the mean (S.E.M). Comparison between the treatment groups and negative control was carried out using one-way analysis of variance (**ANOVA**) followed by Dunnet post hoc test. Analysis and data presentation was done using GraphPad Prism version 8.0.2. Results were considered significant when $P < 0.05$.

CHAPTER THREE

RESULTS

3.1 Organoleptic Properties

Aqueous extract of *Pennisetum pedicellatum* (PPAE)

Colour: Reddish brown

Smell: Weak, woody, earthy smell

Aqueous extract of P*ennisetum pedicellatum* and *Sorghum bicolor* (PPSB)

Colour: Reddish brown

Smell: Weak, woody, earthy smell

3.2 Phytochemical screening

The phytochemical screening of PPAE and PPSB showed the presence of the following constituents: a carbohydrate with reducing sugars, alkaloids, saponins and tanins (Table 2).

Table 2: Phytochemical constituents of PPAE and PPSB.

Phytochemicals	Inference	
	PPAE	PPSB
Carbohydrates	+	+
Reducing sugars	+	+
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Flavonoids	-	+
Phenol	+	+

Key: (-) = Not detected, (+) = Present.

PPAE= Aqueous extract of *Pennisetum pedicellatum*

PPSB= Aqueous extract of *Pennisetum pedicellatum* and *Sorghum bicolor*

3.3 Acute toxicity study

In the acute toxicity studies, no death nor any observable symptoms of toxicity was recorded during the treatment period at all doses of PPAE and PP/SB administered. The animals were apparently healthy with no sign of toxicity up to the dose of 5000 mg/kg. Thus, LD50 was more than 5,000 mg/kg.

3.4 Ethanol-induced ulceration

Intense and widespread thickened gastric lesions of the mucosa were evident in control rats that received 1 ml of absolute ethanol. PPAE showed a significant inhibition of ulceration at 100 mg/kg (****$p<0.001$) and 200 mg/kg (*$p<0.05$) evidenced by reduced ulcer index. This is suggestive of anti-ulcer activity. PPSB at 100 mg/kg showed significant reduction in the ulcer index (*$p<0.05$) also suggestive of anti ulcer activity. Ranitidine (100 mg/kg) caused significant inhibition of ulceration (****$p<0.0001$) showed by a significant reduction in ulcer index (Table 3).

Table 3: Effect of PPAE and PPSB on ethanol-induced ulceration in rats.

Groups	Dose (mg/kg)	Ulcer index (UI)	Inhibition (%)
Control	-	19.40±2.23	-
PPAE	100	8.40±1.03***	56.70
	200	12.20±1.88*	37.11
	500	13.00±2.07	32.99
PPSB	100	12.40 ± 1.60*	36.08
	200	13.00±2.00	32.99
	500	15.40±1.05	20.62
Ranitidine	100	5.00±0.63****	74.23

Values are expressed as Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significantly different from control.

3.5 Indomethacin-induced ulceration

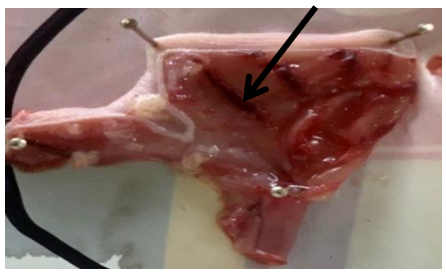
Table 4 shows the effect of the extracts on indomethacin-induced ulceration. There was significant difference between ranitidine (****$p<0.0001$) in ulcer index when compared to the control indicative of anti-ulcer activity. There was also significant reduction in ulceration by PPAE at 100 mg/kg (****$p<0.0001$) and 200 mg/kg (****$p<0.0001$) evidenced by the ulcer index when compared to the control suggestive of anti-ulcer activity. PP/SB showed a significantly inhibit ulceration at 100 mg/kg (****$p<0.0001$), 200 mg/kg (***$p<0.001$) and 500 mg/kg (***$p<0.001$) shown by the ulcer index when compared to the control suggestive of anti-ulcer activity(Table 4).

Table 4: Effect of PPAE and PPSB on Indomethacin-induced ulceration in rats.

Groups	Dose (mg/kg)	Ulcer index (UI)	Inhibition (%)
Control	-	26.80±3.40	-
PPAE	100	5.80±1.36****	78.40
	200	12.00±1.38****	55.20
	500	20.80±1.86	22.40
PPSB	100	14.40 ± 1.86****	46.30
	200	15.20±1.24***	43.30
	500	16.00±1.30***	40.30
Ranitidine	100	12.40±3.56****	53.70

Values are expressed as Mean ± SEM. *p<0.05, ***p<0.001, ****p<0.0001 significantly different from control.

A.



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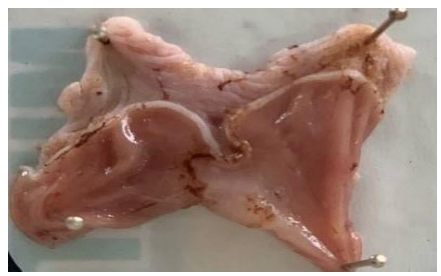

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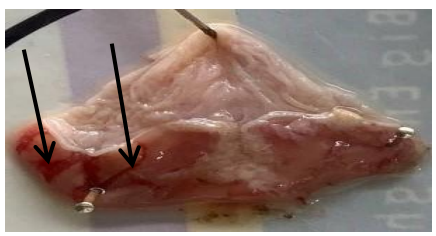

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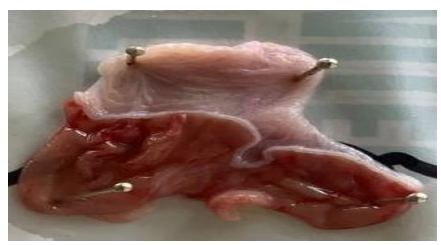


Figure 3: Stomach of rats after Ethanol-induced gastric lesion.

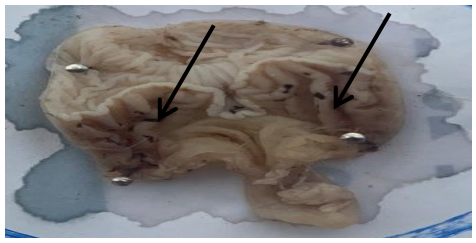
Key: A = Control; B= PPAE (100 mg/kg);

C= PPSB (100 mg/kg); D= Ranitidine (100 mg/kg).

Arrow shows some points of ulceration.

Note the drastic reduction on the pre-treatment with the drug especially with PPAE at a Dose of 100 mg/kg.

A.



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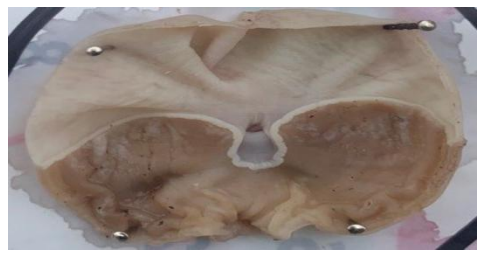

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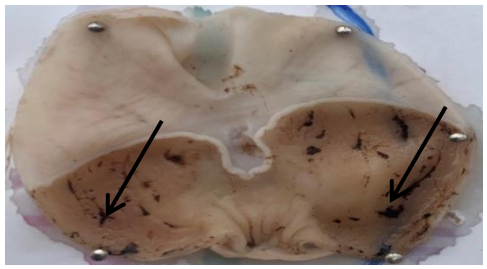

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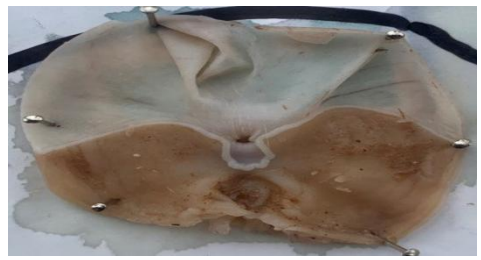

Figure 4: Stomach of rats after Indomethacin-induced gastric lesion.

Key: A = Control; B= PPAE (100 mg/kg);

C= PPSB (100 mg/kg); D= Ranitidine (100 mg/kg).

Arrow shows some points of ulceration.

Note the drastic reduction on the pre-treatment with the drug especially with PPAE at a Dose of 100 mg/kg.

CHAPTER FOUR

DISCUSSION

Peptic ulcer disease is characterized by discontinuation in the lining of the upper gastrointestinal tract where parietal cells secrete pepsin and hydrochloric acid (Henderson and Lander, 1996). It extents into the muscularis propria layer of the gastric and proximal duodenum. Although the exact cause of ulcers is unknown in the majority of instances, it is widely acknowledged that an imbalance between aggressive factors and the endogenous defense mechanism's ability to maintain the integrity of the mucosa leads to ulcer formation (Piper and Stiel, 1986). To regain the balance, different therapeutic agents including medicinal plants are used to inhibit gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production.

The present study was undertaken to evaluate the possible anti-ulcer activity of P*ennisetum pedicellatum* aqueous extract (PPAE) alone and in combination with *Sorghum bicolor (PPSB)* and, in addition, determine its chemical constituents and toxicity profile.

In these ulcer studies, the anti-ulcer activity of the PPAE and PP/SB was evaluated against gastric lesions induced by ethanol and indomethacin, two potent ulcerogens. The results obtained shows PPAE at a dose 100mg/kg has gastro-protective effect in both models of ulcer.

In ethanol-induced ulceration, PPAE showed a significant inhibition of ulceration at 100 mg/kg (****$p<0.001$) and 200 mg/kg (*$p<0.05$) evidenced by reduced ulcer index. This is also evidenced in their percentage inhibition. PPSB also showed a significant reduction in the ulcer index (*$p<0.05$). Ranitidine (100 mg/kg) caused significant inhibition of ulceration (****$p<0.0001$) showed by a significant reduction in ulcer index. Both anti-ulcer effect could

be due to prevention of plasma and cell membrane damage, prevention of disturbance in gastric secretion and gastric mucus depletion, free radical production (Dashputre et al., 2011). Ethanol is a gastric irritant, that has a noxious action on gastric mucosa which causes haemorrhage, necrosis (Lacy and Ito, 1982) and alters permeability to sodium and water leading to cell death and exfoliation of gastric epithelium (Raju et al., 2009).

The mechanism by which the extracts produces its anti-ulcer effect is not clear. However, since stimulation of oxygen derived free radicals (Oates and Hakkinem, 1988) and stimulation of histamine and serotonin release from mast cells (Alarcon et al., 1997) have been implicated in ethanol-induced ulceration, it is probable that the cytoprotective effect of the extracts resides in its ability to produce protective antioxidants that scavenge reactive oxygen species as well as the ability to inhibit mast cell secretory products.

For indomethacin induced ulcer, from the experiment carried out, there was significant difference between ranitidine (****$p<0.0001$) in ulcer index when compared to the control indicative of anti-ulcer activity. There was also significant reduction in ulceration by PPAE at 100 mg/kg (****$p<0.0001$) and 200 mg/kg (****$p<0.0001$) evidenced by the ulcer index when compared to the control suggestive of anti-ulcer activity. PP/SB showed a significantly inhibit ulceration at 100 mg/kg (****$p<0.0001$), 200 mg/kg (***$p<0.001$) and 500 mg/kg (***$p<0.001$) shown by the ulcer index. This anti-ulcer activity could be due to prostaglandin synthensis because indomethacin causes ulceration via suppression of prostaglandin-mediated protective effect (Whittle, 2002). However, anti-ulcer activity of PPAE could also be due to blockade of Histamine H_2 receptor located in the gastric parietal cells, thereby inhibiting the activity of histamine (MacFarlane, 2018). Since indomethacin causes ulceration via suppression of prostaglandin mediated protective functions through inhibition of cyclooxygenase enzyme (Laine et al., 2008). Prostaglandins are known to protect gastric mucosal cells against injury caused by indomethacin (Robert, 1975; Whittle, 1977), as they

regulate the secretion of bicarbonate and mucus, thereby inhibiting gastric acid secretion and maintaining epithelial cell restoration and mucosal blood flow. It also reduces activation of neutrophils and local release of reactive oxgen species (ROS). It is possible that the PPAE stimulates the production of endogenous prostaglandins.

The PPAE showed a better inibition to ulceration when compared to PPSB in both the ethanol induced model and indomethacin induced model at all the doses (100, 200 mg/kg). The PPSB showed a better inhibition to ulceration at 100,200 and 500 mg/kg in the indomethacin induced model compared to that of ethanol-induced model.

The better protective effect seen in indomethacin-induced ulceration as against ethanol-induced, suggests that the PPAE may act mainly by stimulating the synthesis of endogenous prostaglandins.

Reduction in ulcer index (in both models) following administration of the PPAE before the ulcerogen demonstrates the ability of the PPAE to protect the gastric mucosa against ulcer.

The phytochemical screening of the *Pennisetum pedicellatum* aqueous extract (PPAE) alone and in combination with *Sorghum bicolor* (PPSB) revealed the presence of alkaloids, saponins, tannins, reducing sugars, carbohydrates and phenolics.

These result supports earlier report by O.A Ojo, A.B Ojo, M. Barnabas et al., 2021 for aqueous extract of P*ennisetum pedicellatum* alone.

In the acute toxicity study, administration of the PPAE and PPSB orally up to 10mg/kg showed no mortalities. However, the dose was increased up to 5000mg/kg and this still showed no mortalities after 24 hours and 48 hours. This is an indication that the PPAE and PPSB given orally has a relatively wide margin of safety. The PPAE and PPSB is safe at a dose level of 5,000 mg/kg, and the LD_{50} is considered to be >5,000 mg/kg. Any

pharmaceutical drug or compound with an oral LD_{50} higher than 1,000 mg/kg could be considered safe and low toxic (Kennedy et al., 1986). This suggests that PPAE and PPSB is practically non-toxic in a single dose of level 5,000 mg/kg body weight. This study provides essential data on the acute toxicity profile of PPAE and PPSB that should be very useful for any future in vivo and clinical study of this plant medicine. These results showed that using PPAE and PPSB is safe and explained the extensive use of the plant as a traditional medicine in the Northern part of Nigeria. However, to get a more comprehensive toxicity profile of the plant extract, further research on the effect of plant extract on serum biochemical parameters, haematological parameters, and specific organs should be carried out.

CHAPTER FIVE

CONCLUSIONS

The aqueous extract of *Pennisetum pedicellatum* (PPAE) alone and in combination with *Sorghum bicolor* (PPSB) was shown to be non-toxic to a dose of 5 g/kg suggesting that it is relatively safe orally to the rodent used.

Both extracts showed a clear anti-ulcer activity. The anti-ulcer effect observed may be through the production of gatrointestinal prostaglandins, (possibly) free radical scavengers which protect the gastric mucosa and neutralization of stomach acid. More detailed studies are needed to elucidate the exact mechanism by which it produces its effects.

These study revealed that the aqueous extract of *Pennisetum pedicellatum* (PPAE) alone and in combination with *Sorghum bicolor* (PPSB) contains alkaloids, saponins, tannins, reducing sugars, carbohydrates and phenolics. Exhaustive fractionation of the extract will, therefore, be necessary to isolate the active principle responsible for the observed activity.

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