GASTROPROTECTIVE EFFECT OF METHANOL EXTRACT OF *CHASMANTHERA DEPENDENS* ON ASPIRIN INDUCED ULCERATED RATS

BY

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CERTIFICATION

We the undersigned hereby certify that **Mr. AIGBOGUN ENEHIZENA** carried out this work, in the department of Medical Biochemistry, University of Benin, Benin city and we approve same as adequate in scope and quality for the award of Bachelors of science degree (B.Sc) in Medical Biochemistry.

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DEDICATION

I dedicate this work to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on his wings only have I soared.

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This work wouldn't have been possible without the financial and mental encouragement from my parents, Mr and Mrs. Aigbogun

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ABSTRACT

Chasmanthera dependens stem is used in African traditional medicine as a remedy for various maladies and also as a as a remedy for leprosy and lupus; however, scientific evidence to validate its uses in gastric ulcer healing is lacking. This study investigated the 'Gastroprotective effect of methanol extract of *Chasmanthera dependens* on aspirin induced ulcerated rats. A total number of twenty-five (25) albino Wistar rats weighing between (120g-200g) were used in the gastroprotective screening. The rats were randomly divided into three (3) control groups (n=15) and two (2) test groups (n=10) which are classified as follows; Normal control (n=5): Administered only clean water and commercial feed, Negative control (n=5): Aspirin (300mg/kg) body weight. Positive control (n=5): Misoprostol (20mg/kg) body weight for seven (7) days + Aspirin (300mg/kg) body weight, Gastroprotective (n=5): *Chasmanthera dependens* extract (250mg/kg) body weight for seven (7days) + Aspirin (300mg/kg) body weight, Gastroprotective (n=5): *Chasmanthera dependens* extract (500mg/kg) body weight + Aspirin (300mg/kg) body weight. The animals were fasted for a period of 24hours, 300mg/kg body weight dose of aspirin was used to induce ulcer. After four (4) hours, under light anaesthesia by chloroform, the animals were sacrificed, stomach removed, washed, opened on greater curvature and examined for ulceration.Gastric injuries, ulcer index, pH and acid output were evaluated. The results obtained revealed gastric mucosa damage as evident by marked morphological changes, increased ulcer score, ulcer index and acid output at $p< 0.05$. Results of this study showed that methanol can effectively extract the active constituents responsible for anti-ulcerogenic properties. This result has therefore justified the use of extracts of roots of *Chasmanthera dependens* in the traditional treatment of gastric ulcers in Nigeria.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF RESEARCH STUDY

There has been an appreciable increase in research on bioactivity of natural products. The biological aspects most researched are antimicrobial, molluscicidal, insecticidal, parasitic, toxicity tests, anti-tumour, anti-diabetic, antioxidants and a host of others. The isolation and characterization of natural products from African medicinal plants without any biological or pharmacological testing has yielded numerous compounds of novel structure and constitutes the majority of all the recent publications on African Medicinal Plants (Sofowora *et al.,* 1998). Our research is on the plant *Chasmanthera dependens* and our topic of focus is 'Gastroprotective effect of methanol extract of *Chasmanthera dependens* on aspirin induced ulcerated rats.

Ulcers are sores that are slow to heal or keep returning. They can take many forms and can appear both on the inside and the outside of your body. They can be found on places of your body you can see, such as a leg ulcer found on the skin, or in places you can't see, such as a peptic ulcer in the lining of your stomach or upper intestine. From your eye to your foot, you can get them just about anywhere on your body. Injuries, diseases, and infections can cause them. What they look like depends on where you have them and how you got them. While some go away on their own, others cause serious problems if you don't treat them. Digestion occurs more efficiently in the presence of acid, but occurs very well indeed when acid is suppressed. Most commonly **ulcers** occur in either the stomach or duodenum (first part of the small intestine). Symptoms usually manifest as pain or burning in your mid to upper abdomen just below the center of your chest. There are two different **types** of peptic **ulcers**. They are: Gastric **ulcers**, which form in the lining of the stomach. Duodenal **ulcers**, which form in the upper small intestine.Peptic **ulcers can affect** anywhere in the digestive system. Symptoms include stomach pain, sometimes feeling like indigestion, and nausea. Causes include bacteria and certain types of medication. Treatments include proton pump inhibitors (PPIs) and antibiotics. Left untreated, peptic **ulcers can** result in: Internal bleeding. Bleeding **can** occur as slow blood loss that leads to anemia or as severe blood loss that may require hospitalization or a blood transfusion. Severe blood loss may **cause** black or bloody vomit or black or bloody stools. There are various methods and solutions available to treat stomach ulcers, but still

more effective methods are still needed for the treatment of ulcer, hence the importance of our research study.

Our research plant is *Chasmanthera dependens*, the plant is a woody climber with a rough stem. The young stem is hairy. The leaves are oval or rounded, 3-10 cm long and about the same size in width. The leaf surface is papery and sparsely hairy on both sides. It has a heart-shaped base, and the apex is very shortly drawn out; the stalks are leafy and up to 30 cm long. The plant produces many flowers in the axils of the leaves; they are tiny and hairy and borne on long, slender common stalks. The plant contain nonnitrogenous bitter principles such as chasmanthin, columbin, and palmatine. Berberine-type alkaloids, palmatine, colombamine, and jateorhizine also occur in the plant (Onabanjo *et al*., 1991), it is contains other compound like alkaloids, The roots contain berberine, which is reported to control leishmaniasis (Burkill *et al*., 1997). Methanol extracts of the dried leaves have shown significant analgesic and anti-inflammatory effects (Onabanjo *et al*., 1990). The fundamental motivation behind this examination is to decide the impact of chasmanthera depemdens on ulcer levels.

1.2 AIM OF RESEARCH; The major aim of this research is to ascertain and provide scientific information on the methanol extracts of Chasmanthera dependes to acertain its gastroprotective effect on aspirin induced ulcerated rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 WHAT IS GASTRIC ULCER

Gastric ulcer is a sore that develops on the lining of the oesophagus, stomach or small intestine. Ulcers occur when stomach acid damages the lining of the digestive tract. Common causes include the bacteria H. Pylori and anti-inflammatory pain relievers including aspirin. Gastric ulcers, which are also known as gastric ulcers, are painful sores in the stomach lining. Gastric ulcers are a type of peptic ulcer disease. Gastric ulcers are any ulcers that affect both the stomach and small intestines. Stomach ulcers occur when the thick layer of mucus that protects your stomach from digestive juices is reduced. This allows the digestive acids to eat away at the tissues that line the stomach, causing an ulcer. Known damaging factors of the barrier include;

1. Smoking - Cigarette smoking appears to be a risk factor for the development, maintenance, and recurrence of peptic ulcer disease. Smoking has an inconsistent effect on gastric acid secretion, but it does have other effects on upper gastrointestinal function that could contribute to the pathogenesis of peptic ulcer disease (Arthur and John, 2000). These include;

(a) Interference with the action of histamine-2 antagonists,

(b) Acceleration of gastric emptying of liquids,

(c) Promotion of duodenogastric reflux,

(d) Inhibition of pancreatic bicarbonate secretion,

(e) Reduction in mucosal blood flow, and

(f) Inhibition of mucosal prostaglandin production.

Because these effects are related directly to the act of smoking and cessation of smoking is associated with the prompt recovery of the respective functions, smokers will benefit immediately by stopping or reducing cigarette consumption (John *et al*., 1989).

2. Alcohol - Overtime alcohol wears down the linings of the stomach and intestine, hence it is a predisposing cause of acute and haemorrhagic gastric erosion in humans (Debashis *et al*., 2002). Ethanol lowers the concentration of non-protein sulphydryls especially glutathione (Szabo *et al*., 1981), thereby exerting ulcerogenic effect by increasing oxygen reactive species formation (Pihan and Szelenzyl, 1985).

3. Helicobacter pylori infection - H. pylori cause gastritis, a condition that involves inflammation of the stomachs lining. H. pylori infection is also linked to stomach cancer; however, the American Cancer Society states that most people with H. pylori in their stomach never develop stomach cancer (Walsh *et al*., 1985). The stomach has a layer of mucus that is designed to protect it from stomach acid (Parsonnet *et al*., 1994). H. pylori attack this mucus lining and leave part of the stomach exposed to acid (Walsh and Peterson, 1995). Together, the bacteria and the acid can irritate the stomach, causing ulcers, gastritis, or stomach cancer. Two-thirds of the world's population have H. pylori, according to the Centers for Disease Control and Prevention (CDC).

4. Stress - Stress is also a major cause of gastric ulcer (Barocelli *et al*., 1997). Stress as implicated in the development of ulcer consists of physical and emotional stresses. Emotional stress may make an ulcer more difficult to heal and more painful but the stress itself does not cause an ulcer (Cho *et al*., 1992).

Others include; Ischemia, Hypoxia, Hydrochloric acid.

2.1.2 ETIOLOGY

The most common etiologies of gastric ulcers include a bacterial infection with Helicobacter pylori and gastric prostaglandin loss associated with the use of non-steroidal anti-inflammatory medications. Less common etiologies include hypergastrinemia (Zollinger-Ellison syndrome), viral infections such as CMV, chemotherapy and radiation, gastric outlet obstruction, gastric infiltrative disorders such as malignancy, cigarette smoking, and Crohn disease. The common

factor in all of these etiologies is that they promote a breakdown in the mucosal barrier and expose the gastric mucosa to the damaging effects of acid (Scida *et al*., 2018).

2.1.3 EPIDEMIOLOGY

Gastric ulcers are a part of peptic ulcer disease, which carries a lifetime prevalence of 5 to 10% of patients, which is likely an underestimation of the disease as some patients may remain asymptomatic. (WHO, 2019) Studies have shown that the prevalence of gastric ulcers increases with age and with the chronicity of NSAID use. Research shows that smoking leads to a relative risk of 2.0 times that of non-smokers for developing gastric ulcers (The green institute, 2020). There is no difference between men and women in the prevalence of gastric ulcers. The prevalence of Helicobacter pylori infection at age 60 approximates 50% in the US population. Estimates are that 25% of chronic NSAID users will develop gastric ulcers (Kayali *et al*., 2018).

2.1.4 HISTOPATHOLOGY

On histopathology, one will see an ulcer base with clear margins that penetrates the muscularis propria and into the submucosa. Inflammatory debris on the epithelial surface is often present. In the submucosa, one will see fibrosis and thickened blood vessels (Barchi *et al*., 2011).

2.1.5 PATHOPHYSIOLOGY

The pathophysiology of gastric ulcer development depends on the insult. Since about 80 to 90% of gastric ulcers result from either Helicobacter pylori and/or NSAID use, (Graham et al., 2019) a detailed discussion will focus each in detail, first regarding Helicobacter pylori - these bacteria colonize about 45-50% of the stomach mucosa worldwide. It is a bacterium that people are inoculated with at an early age, especially in developing countries with lower socioeconomic status and crowded households, these bacteria induce an inflammatory response in the host that leads to an epithelial response, degeneration, and injury, known as gastritis, typically, patients with this infection develop pan-gastritis (Graham *et al*., 2019). This damages the antral somatostatin release, which leads to an increase in gastrin secretion which stimulates increased acid production. Patients who develop gastric ulcers are those in whom the bacteria has remained in the antrum. Parietal cells of the more proximal gastric body still have full production capabilities preventing ulcer genesis in this area. A common bacterial virulence factor is the

production of cagA, which leads to more cytokine cell destruction and mucosal damage (Barch *et al*., 2011).

NSAID medications are the other most common etiology causing gastric ulcers. (Cooper *et al*., 2005) There are multiple mechanisms by which NSAID medications lead to ulceration. The drugs themselves are weak acids when they become exposed to gastric acid. They remain in the epithelial cells and lead to increased cellular permeability, which leads to physical cellular injury. NSAIDs inhibit the cyclooxygenase-1 enzyme, which usually increases prostaglandin synthesis which in turn leads to gastric bicarbonate secretion, mucus barrier formation, increased mucosal blood flow, and accelerated epithelial cell restitution and repair after injury or cell death.(Paul-Clark et al., 2005) NSAID medications allow the gastric mucosa to become more vulnerable to gastric acid and pepsin damage (Dore and Violi, 2018) Overall, the most harmful physiological damage results from the decrease in gastric blood flow and the mild ischemia it causes in the gastric mucosa. Overall, the pathophysiology of gastric ulcer development depends on the etiology, but they all lead to the loss or damage of the gastric mucosal integrity (Leondro *et al*., 2018).

2.1.6 Acid secretion

Acid is secreted by parietal cells in the proximal two thirds (body) of the stomach. Gastric acid aids digestion by creating the optimal pH for pepsin and gastric lipase and by stimulating pancreatic bicarbonate secretion (John *et al*., 1989). Acid secretion is initiated by food: the thought, smell, or taste of food effects vagal stimulation of the gastrin-secreting G cells located in the distal one third (antrum) of the stomach (Arthur and john, 2002). The arrival of protein to the stomach further stimulates gastrin output. Circulating gastrin triggers the release of histamine from enterochromaffin-like cells in the body of the stomach (Bigheti *et al*., 2005). Histamine stimulates the parietal cells via their H2 receptors (Rodger *et al*., 1992) The parietal cells secrete acid, and the resulting drop in pH causes the antral D cells to release somatostatin, which inhibits gastrin release (negative feedback control) (Russell and Norman, 1993).

2.1.7 Mucus

The mucus layer is the first line of defense against infiltration of microorganisms, digestive enzymes and acids, digested food particles, microbial by-products, and food-associated toxins. This layer coats the interior surface of the GI tract, lubricates luminal contents and acts as a physical barrier to bacteria and other antigenic substances present in the lumen. The moist, nutrient-rich mucus layer adjacent to the epithelial barrier of the GI tract is also essential in the maintenance of intestinal homeostasis and contains a thriving biofilm including beneficial and pathogenic microbial populations (Pearsaon *et al*., 1980). Emerging evidence demonstrates changes in the gut-brain axis in neurological disease involving the enteric nervous system located within the wall of the GI tract. Interestingly, mucus production is regulated by molecular pathways involved in developmental processes and nervous system activity. Multiple neurological disorders present with gastrointestinal dysfunction and microbial dysbiosis but whether alterations in mucus structure and function are driving these changes is unknown. Therefore, we propose that alterations in enteric nervous system function and mucus production may occur in neurological disease and contribute to GI symptoms and dysbiosis (Ermund *et al*., 2013).

2.1.8 PROSTAGLANDINS

Prostaglandins are a family of chemicals that are produced by the cells of the body and have several important functions. They promote inflammation that is necessary for healing, but also results in pain, and fever; support the blood clotting function of platelets; and protect the lining of the stomach from the damaging effects of acid. Prostaglandins are produced within the body's cells by the enzyme cyclooxygenase (COX). There are two COX enzymes, COX-1 and COX-2. Both enzymes produce prostaglandins that promote inflammation, pain, and fever. However, only COX-1 produces prostaglandins that support platelets and protect the stomach. Nonsteroidal anti-inflammatory drugs (NSAIDs) block the COX enzymes and reduce prostaglandins throughout the body. As a consequence, ongoing inflammation, pain, and fever are reduced. Since the prostaglandins that protect the stomach and support platelets and blood clotting also are reduced, NSAIDs can cause ulcers in the stomach and promote bleeding (Lanas *et al*., 2017)

2.1.9 Non–Steriodal Anti-inflammatory Drugs (NSAIDs) NSAIDS

Nonsteroidal anti-inflammatory drugs (NSAIDs) are members of a drug class that reduces pain, decreases fever, prevents blood clots, and in higher doses, decreases inflammation. Side effects depend on the specific drug but largely include an increased risk of gastrointestinal ulcers and bleeds, heart attack, and kidney disease (Bally and Lanas, 2017). The term nonsteroidal distinguishes these drugs from steroids, which while having a similar eicosanoid-depressing, anti-inflammatory action, have a broad range of other effects. First used in 1960, the term served to distance these medications from steroids, which were particularly stigmatized at the time due to the connotations with anabolic steroid abuse (Buer *et al*., 2014). NSAIDs work by inhibiting the activity of cyclooxygenase enzymes (COX-1 or COX-2). In cells, these enzymes are involved in the synthesis of key biological mediators, namely prostaglandins, which are involved in inflammation, and thromboxanes, which are involved in blood clotting. There are two types of NSAIDs available: non-selective and COX-2 selective (Graham *et al*., 2004). Most NSAIDs are non-selective and inhibit the activity of both COX-1 and COX-2. These NSAIDs, while reducing inflammation, also inhibit platelet aggregation (especially aspirin) and increase the risk of gastrointestinal ulcers/bleeds (Graham *et al*., 2004). COX-2 selective inhibitors have less gastrointestinal side effects but promote thrombosis and some of these agents substantially increase the risk of heart attack. As a result, certain older COX-2 selective inhibitors are no longer used due to the high risk of undiagnosed vascular disease (Graham *et al*., 2004). These differential effects are due to the different roles and tissue localisations of each COX isoenzyme, (Day *et al*., 2004). By inhibiting physiological COX activity, all NSAIDs increase the risk of kidney disease (Brater *et al*., 2003) and through a related mechanism, heart attack (Bleumink *et al*., 2003). In addition, NSAIDs can blunt the production of erythropoietin resulting in Anaemia, since Haemoglobin needs this hormone to be produced. Prolonged use is dangerous and case studies have shown the health risk with celecoxib.

The most prominent NSAIDs are aspirin, ibuprofen, and naproxen, all available over the counter (OTC) in most countries (Warden *et al*., 2010). Paracetamol (acetaminophen) is generally not considered an NSAID because it has only minor anti-inflammatory activity. Acetaminophen treats pain mainly by blocking COX-2 and inhibiting endocannabinoid reuptake almost exclusively within the brain, but not much is in the rest of the body (Hinz and Clive, 2008).

Mechanism of action - Enzyme inhibitor

Biological target - COX-1 and COX-2

2.2 TYPES OF GASTRIC ULCER

Gastric ulcers is divided based on the basis of location (Johnson classification) and endoscopic findings (Sakita classification):

1. JOHNSON CLASSIFICATION

Gastric ulcer is further classified into 3 subtypes depending upon their location:

- Type 1: Ulcer present at the body of stomach without involving duodenum, pylorus or prepyloric region and not associated with hypersecretion of gastric acid, (Johnson *et al*., 1965).
- Type 2: Ulcer present at the body of stomach combined with duodenum and associated with gastric acid hypersecretion, (Baron *et al.,* 1963).
- Type 3: Ulcer close to pylorus and associated with gastric acid hypersecretion, (Johnson *et al*., 1955).

2. SAKITA CLASSIFICATION

Gastric ulcer classification by using endoscopic staging system of Sakita into three stages:

Active, healing and scarring, (kaneko *et al*., 2000).

(A) ACTIVE

Active stage includes;

- A1. Surrounding mucosa is found to be edematously swollen and there is no regenerating epithelium seen on endoscopy (Baron *et al*., 1963).

- A2. Surrounding mucosa is less edematous, a small amount of regenerating epithelium is seen at the ulcer margin. A red halo in the marginal zone, a white slough circle and converging mucosal folds into the ulcer margin are frequently seen (Johnson *et al*., 1965).

(B) HEALING

Healing stage includes;

- H1. The white coating is becoming thin and the regenerating epithelium is extending into the ulcer base. The gradient between the ulcer margin and the ulcer floor is becoming flat. The ulcer crater is still evident and the margin of the ulcer is sharp. The diameter of the mucosal defect is about one-half to two thirds that of A1 (Kaneko *et al*., 2000)

- H2. The defect is smaller than in H1 and the regenerating epithelium covers most of the ulcer floor. The area of white coating is about a quarter to one-third that of A1 (Baron *et al*., 1963)

(C) SCARRING

Scarring stage includes;

- S1. The regenerating epithelium completely covers the floor of the ulcer. The white coating has disappeared. Initially, the regenerating region is markedly red but upon close observation, many capillaries can be seen and this is called ‘‘red scar’’ (Tytgat *et al*., 2011).

- S2. In several months to a few years, the redness is reduced to the color of the surrounding mucosa and this is called ‘‘white scar’’ (Jonson *et al*., 1965).

2.3 CAUSES OF GASTRIC ULCER

Your stomach normally produces acid to help with the digestion of food and to kill germs (bacteria). This acid is corrosive, so some cells on the inside lining of the stomach and the first part of the gut (small intestine) known as the duodenum produce a natural mucous barrier. This protects the lining of the stomach and duodenum. There is normally a balance between the amount of acid that you make and the mucous defense barrier. An ulcer may develop if there is

an alteration in this balance, allowing the acid to damage the lining of the stomach or duodenum (Arthur *et al*., 2000). Causes include:

2.3.1 A BACTERIUM

Helicobacter pylori bacteria commonly live in the mucous layer that covers and protects tissues that line the stomach and small intestine. Often, the H. pylori bacterium causes no problems, but it can cause inflammation of the stomach's inner layer, producing an ulcer. It's not clear how H. pylori infection spreads. It may be transmitted from person to person by close contact, such as kissing. People may also contract H. pylori through food and water (Baena *et al*., 2002)

2.3.2 ANTI-INFLAMMATORY MEDICINES

Anti-inflammatory medicines including aspirin are sometimes called non-steroidal anti-inflammatory drugs (NSAIDs). Taking aspirin, as well as certain over-the-counter and prescription pain medications called nonsteroidal anti-inflammatory drugs (NSAIDs), can irritate or inflame the lining of your stomach and small intestine. These medications include ibuprofen (Advil, Motrin IB, others), naproxen sodium (Aleve, Anaprox DS, others), ketoprofen and others. They do not include acetaminophen (Tylenol, others) (Laurence *et al*., 2020).

2.3.3 USE OF MEDICATIONS

Taking certain other medications along with NSAIDs, such as steroids, anticoagulants, low-dose aspirin, selective serotonin reuptake inhibitors (SSRIs), alendronate (Fosamax) and risedronate (Actonel), can greatly increase the chance of developing ulcers (Caswell *et al*., 1992)

Other causes of gastric ulcers may include;

2.3.4 Crohn's disease.

2.3.5 Viral infection.

2.3.6 Stomach cancer.

RISK FACTORS

In addition to having risks related to taking NSAIDs, you may have an increased risk of peptic ulcers if you:

- Smoke. Smoking may increase the risk of peptic ulcers in people who are infected with H. pylori (Yuda *et al*., 1993).
- Drink alcohol. Alcohol can irritate and erode the mucous lining of your stomach, and it increases the amount of stomach acid that's produced (Mohammed *et al*., 1994).
- Have untreated stress.
- Eat spicy foods.

Alone, these factors do not cause ulcers, but they can make ulcers worse and more difficult to heal.

DIAGNOSIS OF GASTRIC ULCER DISEASE

Gastric ulcer disease can be diagnosed by some symptoms and signs experienced by individual patients. Other ways of diagnosis include; physical examination, laboratory studies and methods of visualization of ulcers like endoscopy.

2.4 SIGNS AND SYMPTOMS

The main symptom caused by a stomach ulcer is having a pain in the upper abdomen. Other symptoms may include:

2.4.1 Perforation - This is the term used to describe the ulcer having gone all the way through (perforated) the wall of the stomach. Food and acid in the stomach then leak out of the stomach. This usually causes severe pain and makes you very unwell. Stomach perforation is a medical emergency and needs hospital treatment as soon as possible.

2.4.2 Stomach blockage - This is now rare. An ulcer at the end of the stomach can cause the outlet of the stomach (the part of the stomach that goes into the duodenum) to narrow and cause an obstruction. This can cause frequent severe vomiting.

2.4.3 Bloating - This means your tummy swells because your stomach is full of gas or air.

2.4.4 Retching - Also known as 'heaving'. This means sounding and looking as though you're about to be sick (vomit) but not actually vomiting.

2.4.5Feeling sick (nausea)

2.4.6 Vomiting

2.4.7 Unexplained weigh loss

2.4.8 Trouble breathing

2.4.9 Bleeding - Dark blood in stools, or stools that are black or tarry (Walsh *et al*., 1992).

2.4.9 Appetite changes.

2.4.9 Dyspepsia - Indigestion is often a sign of an underlying problem, such as gastroesophageal reflux disease (GERD), ulcers, or gallbladder disease, rather than a condition which is known as dyspepsia, dyspepsia is a persistent or recurrent pain or discomfort in the upper abdomen (Devauli and Castelli, 1999).

2.5 COMPLICATIONS

Left untreated, gastric ulcers can result in;

- Internal bleeding - Bleeding can occur as slow blood loss that leads to anemia or as severe blood loss that may require hospitalization or a blood transfusion. Severe blood loss may cause black or bloody vomit or black or bloody stools.

- A hole (perforation) in your stomach wall - Peptic ulcers can eat a hole through (perforate) the wall of your stomach or small intestine, putting you at risk of serious infection of your abdominal cavity (peritonitis).

- Obstruction - Peptic ulcers can block passage of food through the digestive tract, causing you to become full easily, to vomit and to lose weight either through swelling from inflammation or through scarring.
- Gastric cancer - Studies have shown that people infected with H. pylori have an increased risk of gastric cancer.

2.6 PREVENTION OF GASTRIC ULCER

You may be able to reduce the risk of gastric ulcer by following these few steps;

a. Protect yourself from infections. It's not clear just how H. pylori spreads, but there's some evidence that it could be transmitted from person to person or through food and water. You can take steps to protect yourself from infections, such as H. pylori, by frequently washing your hands with soap and water and by eating foods that have been cooked completely (Barch *et al*., 2018).

b. Use caution with pain relievers. If you regularly use pain relievers that increase your risk of peptic ulcer, take steps to reduce your risk of stomach problems. For instance, take your medication with meals. Work with your doctor to find the lowest dose possible that still gives you pain relief. Avoid drinking alcohol when taking your medication, since the two can combine to increase your risk of stomach upset. If you need an NSAID, you may need to also take additional medications such as an antacid, a proton pump inhibitor, acid blocker or cytoprotective agent. A class of NSAIDs called COX-2 inhibitors may be less likely to cause peptic ulcers, but may increase the risk of heart attack (Mario *et al*., 2018).

2.7 GASTRIC ULCER TREATMENT

Gastric ulcer have become much less common since the 1980s because of much more effective treatments. So people with stomach ulcers now usually get better much more quickly.

2.7.1 A general improvement in our lifestyle can improve symptoms, such as;

i. Lose weight if you are overweight

ii. Avoid any trigger foods, such as coffee, chocolate, tomatoes, fatty foods or spicy foods.

iii. Restriction of alcohol consumption (Palmer and Penman, 1999).

iv. Eat smaller meals and eat your evening meal 3-4 hours before going to bed.

v. Quit smoking

2.7.2 Acid suppressing medication

A 4- to 8-week course of a medicine that greatly reduces the amount of acid that your stomach makes is usually advised.

2.7.3 Treatment of gastric ulcer caused by Helicobacter pylori (H. pylori)

Most stomach ulcers are caused by infection with H. pylori (Vergara *et al*., 2005). Therefore, a main part of the treatment is to clear this infection. If this infection is not cleared, the ulcer is likely to return once you stop taking acid-suppressing medication. H. pylori is killed by certain antibiotics. However, a combination of medicines is needed to get rid of it completely. This is referred to as combination therapy although because it gets rid of (eradicates) the germ it is also referred to as eradication therapy. (Fuller *et al*., 1991) You need to take two antibiotics at the same time. In addition, you need to take a medicine to reduce the acid in the stomach. This allows the antibiotics to work well in the stomach. You need to take eradication therapy for a week (Fuller *et al*., 1991). It is important to take all the medication exactly as directed and to take the full course.

2.7.4 Treatment of gastric ulcer caused by anti-inflammatory medicine

If possible, you should stop taking the anti-inflammatory medicine. This allows the ulcer to heal. You will also normally be prescribed an acid-suppressing medicine for several weeks, this stops the stomach from making acid and allows the ulcer to heal (Wallace *et al*., 2005). However, in many cases, the anti-inflammatory medicine is needed to ease symptoms of arthritis or other painful conditions, or aspirin is needed to protect against blood clots (David *et al*., 1998). In these situations, one option is to take an acid-suppressing medicine each day indefinitely. This reduces the amount of acid made by the stomach and greatly reduces the chance of an ulcer forming again (Dobrilla *et al*., 1989).

2.7.5 Antibiotics

If you have an H. pylori infection, you'll usually be prescribed a course of 2 antibiotics, which each need to be taken twice a day for a week (Lanas and Serrano, 2000). The antibiotics most commonly used are amoxicillin, clarithromycin and metronidazole.

2.7.6 Proton pumps inhibitors (PPIs)

PpIs work by reducing the amount of acid your stomach produces, preventing further damage to the ulcer as it heals naturally. They're usually prescribed for 4 to 8 weeks. Omeprazole, pantoprazole and lansoprazole are the PPIs most commonly used to treat stomach ulcers (Lindberg *et al*., 1990).

2.7.7 Histamine H2 - receptor antagonist

Like PPIs, H2-receptor antagonists work by reducing the amount of acid your stomach produces. Ranitidine is the most widely used H2-receptor antagonist for treating stomach ulcers (Bertaccini *et al*., 1981).

2.7.8 Antacids and alginates

Taking additional antacid medication to neutralize your stomach acid and provide immediate, but short-term, symptom relief (Arthur and John, 2000). Some antacids also contain a medicine called an alginate, which produces a protective coating on the lining of your stomach. These medications are available to buy over the counter at pharmacies. Your pharmacist can advise on which is most suitable for you. Antacids should be taken when you experience symptoms or when you expect them, such as after meals or at bedtime. Antacids containing alginates are best taken after meals.

2.7.9 Surgery

In the past, surgery was commonly needed to treat a stomach ulcer. This was before it was discovered that H. pylori infection was the cause of most stomach ulcers, and before modern acid-suppressing medicines became available. Surgery is now usually only needed if a complication of a stomach ulcer develops, such as severe bleeding or a hole (perforation).

2.7.9 Use of Herbal products.

WHAT TO DO AFTER TREATMENT

A repeat gastroscopy (endoscopy) is usually advised a few weeks after treatment has finished. This is mainly to check that the ulcer has healed. It is also to be doubly certain that the 'ulcer' was not due to stomach cancer. If your ulcer was caused by H. pylori then a test is advised to check that the H. pylori infection has gone. This is done at least four weeks after the course of combination therapy has finished (Laine *et al*., 2000)

2.8 TEST FOR GASTRIC ULCER

If your doctor thinks you may have a stomach ulcer, the initial tests will include some blood tests. These tests will help to check whether you have become anaemic because of any bleeding from the ulcer. The blood test will also check to see that your liver and pancreas are working properly.

The main tests that are then used to diagnose a stomach ulcer are as follows:

2.8.1 A test to detect the H. pylori germ (bacterium) - is usually done if you have a stomach ulcer. The H. pylori bacterium can be detected in a sample of stool (faeces), or in a 'breath test', or from a blood test, or from a biopsy sample taken during a gastroscopy (Hayley *et al*., 2020).

2.8.2 Gastroscopy (endoscopy) - is the test that can confirm a stomach ulcer. Gastroscopy is usually done as an outpatient 'day case'. You may be given a sedative to help you to relax. In this test, a doctor looks inside your stomach by passing a thin, flexible telescope down your gut (oesophagus). The doctor will then be able to see any inflammation or ulcers in your stomach (American society for Gastrointestinal Endoscopy, 1999).

2.8.3 Small samples (biopsies) - are usually taken of the tissue in and around the ulcer during gastroscopy. These are sent to the laboratory to be looked at under the microscope. This is important because some ulcers are caused by stomach cancer. However, most stomach ulcers are not caused by cancer (Drini *et al*., 2017).

2.8.4 Radiography

2.9. **THE PLANT *CHASMANTHERA DEPENDES***

Fig 1.0; *Chasmanthera dependes*, (The Green Institute, 2020).

Fig 1.1; *Chasmanthera dependes*, (The Green Institute, 2020).

Fig 1.2; *Chasmanthera dependes*, (West African Plants, 2020)

2.9.1 **DESCRIPTION AND ORIGIN**

A woody liane of the dense evergreen or semi-deciduous lowland forest and riparian woodland of the drier guinean zone from Sierra Leone to Nigeria, and in Cameroun across Africa to Ethiopia and Somalia, Kenya, Tanganyika and Zambia, (Oliver *et al*., 1983). The plant is a woody climber with a rough stem. The young stem is hairy. The leaves are oval or rounded, 3-10 cm long and about the same size in width. The leaf surface is papery and sparsely hairy on both sides. It has a heart-shaped base, and the apex is very shortly drawn out; the stalks are leafy and up to 30 cm long. The plant produces many flowers in the axils of the leaves; they are tiny and hairy and borne on long, slender common stalks.

2.9.2 TAXONOMY

Kingdom	Plantae – Plantes, Planta, Vegetal plants.
Subkingdom	Viridiplantae – Green plants.
Infrakingdom	Streptophyta – Land plants.
Superdivision	Embryophyta.
Division	Tracheophyta – Vascular plants, tracheophytes.
Subdivision	Spermatophytina – Spermatophytes, seed plants, phanérogames.
Class	Magnoliopsida.
Superoder	Ranunculales.
Order	Menispermaceae.
Family	Chasmantheroideae.

Genus *Burasaieae.*

Species *Chasmanthera Dependes.*

(Uniprot, 2020).

Common names:

Botanical name - *chasmanthera dependes* (Hochst *et al*., 1844).

Local names include; Igbo (ogbo). Yoruba (ato oloriraun). Bini (sensen). Hausa (ato).

2.9.3 CONSTITUENTS OF *CHASMANTHERA DEPENDES*

Chasmanthera and the other members of the plant family Menispermaceae contain nonnitrogenous bitter principles such as chasmanthin, columbin, and palmatine. Berberine-type alkaloids, palmatine, colombamine, and jateorhizine also occur in the plant (Onabanjo *et al*., 1991). The stem bark of *Chasmanthera dependens* is rich in alkaloids and contains the quaternary protoberberine alkaloids jateorrhizine, palmatine (berbericinine), columbamine, pseudicolumbamine, magnoflorine, and the non-phenolic quaternary alkaloids tetrahydropalmatine, liriodenine, lysicamine (oxonuceferine), O,O-dimethylcorytuberine, anonaine, glaucine, norglaucine, oxoglaucine and nornuceferine (Almeida *et al*., 2001). It also contains the tetrahydroprotoberberine type alkaloids govanine and coreximine, the pavine type alkaloid bisnorargemonine and the morphinandienone type alkaloid pallidine, as well as the furanoid diterpene 8-hydroxycolumbine (Okoli *et al*., 2003). The roots contain berberine, which is reported to control leishmaniasis (Burkill *et al*., 1997). Methanol extracts of the dried leaves have shown significant analgesic and anti-inflammatory effects (Onabanjo *et al*., 1990). Ethanol extracts and crude water extracts of the roots showed significant antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans, Microsporum audonii, Trichoderma viride* and *Trichophyton mentagrophytes* (Morebise *et al*., 2001). The ethanol extracts of the plants were more active than the water extracts (Irvine *et al*., 1961).

2.9.4 ETHNOMEDICINAL USES

The drug is used in both Africa and Asia as a remedy for leprosy and lupus. In East Africa, it has been dispensed for fevers, abdominal distress, and venereal diseases. The Xhosa and the Mfengu of southern Africa use the leaves as a food vegetable (Adekunle *et al*., 2002). The plant is used in West Africa as a fever remedy and as an ingredient in steam treatment of malaria.

In West Africa leaf and stem sap are locally applied to cure sprains and bruises, as a dressing for fractures or mixed with shea butter as an embrocation to treat pain and stiffness. The bark is chewed as a remedy for venereal discharges or as a general tonic for physical or nervous weakness in inflammatory and exhausting diseases. In Nigeria a stem maceration together with stems and roots of several other plants is drunk against convulsions. In Kenya the stem is roasted and eaten to treat convulsions in infants. In Uganda the plant is used against dementia, snakebites and epilepsy. A decoction of freshly pounded roots mixed with roots of Vernonia sp. is drunk to cure malaria. A decoction of pounded roots mixed with leaves of Tagetes sp. is drunk by children to treat cough. In DR Congo the leaf sap is applied as first aid to stop bleeding of wounds. In Nigeria the fibrous stem is beaten and used as a sponge. In Ethiopia Borana pastoralists eat the roots and leaves (Bouquet *et al*., 1974).

2.9.5 MECHANISM OF INDUCTION OF ULCER ASPIRIN (NSAIDS)

Non-steroidal anti-inflammatory drugs including low-dose aspirin are some of the most commonly used medicines (Guigan *et al*., 1991). They are associated with gastrointestinal mucosal injury. Before prescribing, it is important to assess the patient's gastrointestinal risk factors such as age and history of peptic ulcers. Patients at high risk may require co-prescription to reduce the risk of peptic ulcers. A daily dose of a proton pump inhibitor is the most effective method of reducing the risk of ulcers induced by non-steroidal anti-inflammatory drugs (Soll *et al*., 1991).

They have good efficacy and a long history of clinical use, but can cause peptic ulcers which may have fatal complications (Macdonald *et al*., 1988). Given widespread use of NSAIDs and aspirin, the associated gastrointestinal toxicities have substantial implications for the healthcare system (Sneader *et al*., 2000).

2.9.6 ULCERS AND NSAIDS

Peptic ulcer disease is a well-recognized complication of NSAID use. Inhibition of COX-1 in the gastrointestinal tract leads to a reduction of prostaglandin secretion and its cytoprotective effects in gastric mucosa. This therefore increases the susceptibility to mucosal injury, (Levy *et al*., 1978). Inhibition of COX-2 may also play a role in mucosal injury.

ASPIRIN

Also known as Aspirin, acetylsalicylic acid (ASA) is a commonly used drug for the treatment of pain and fever due to various causes. Acetylsalicylic acid has both anti-inflammatory and antipyretic effects. This drug also inhibits platelet aggregation and is used in the prevention of blood clots stroke, and myocardial infarction (MI) Label. Interestingly, the results of various studies have demonstrated that long-term use of acetylsalicylic acid may decrease the risk of various cancers, including colorectal, esophageal, breast, lung, prostate, liver and skin cancer (Alfonso and Bhat, 2014). Aspirin is classified as a non-selective cyclooxygenase (COX) inhibitor, (Vane and Ornelas, 2017) and is available in many doses and forms, including chewable tablets, suppositories, extended release formulations, and others (Hasan *et al*., 2018). Acetylsalicylic acid is a very common cause of accidental poisoning in young children. It should be kept out of reach from young children, toddlers, and infants Label.

Molecular weight of aspirin: Average (180.1574). Monoisotopic (180.042258744) (Dhaghat *et al*., 2007).

Chemical formula - C9 H8 O4

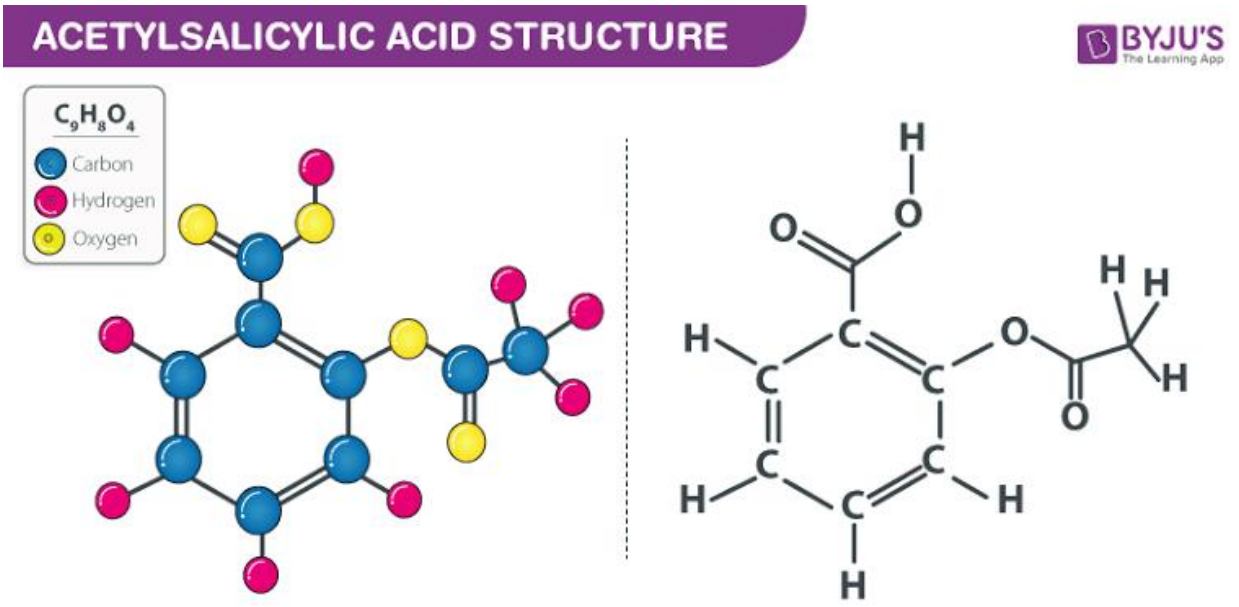

Fig 2.0 Structure of Aspirin, (Snearder *et al*., 2000).

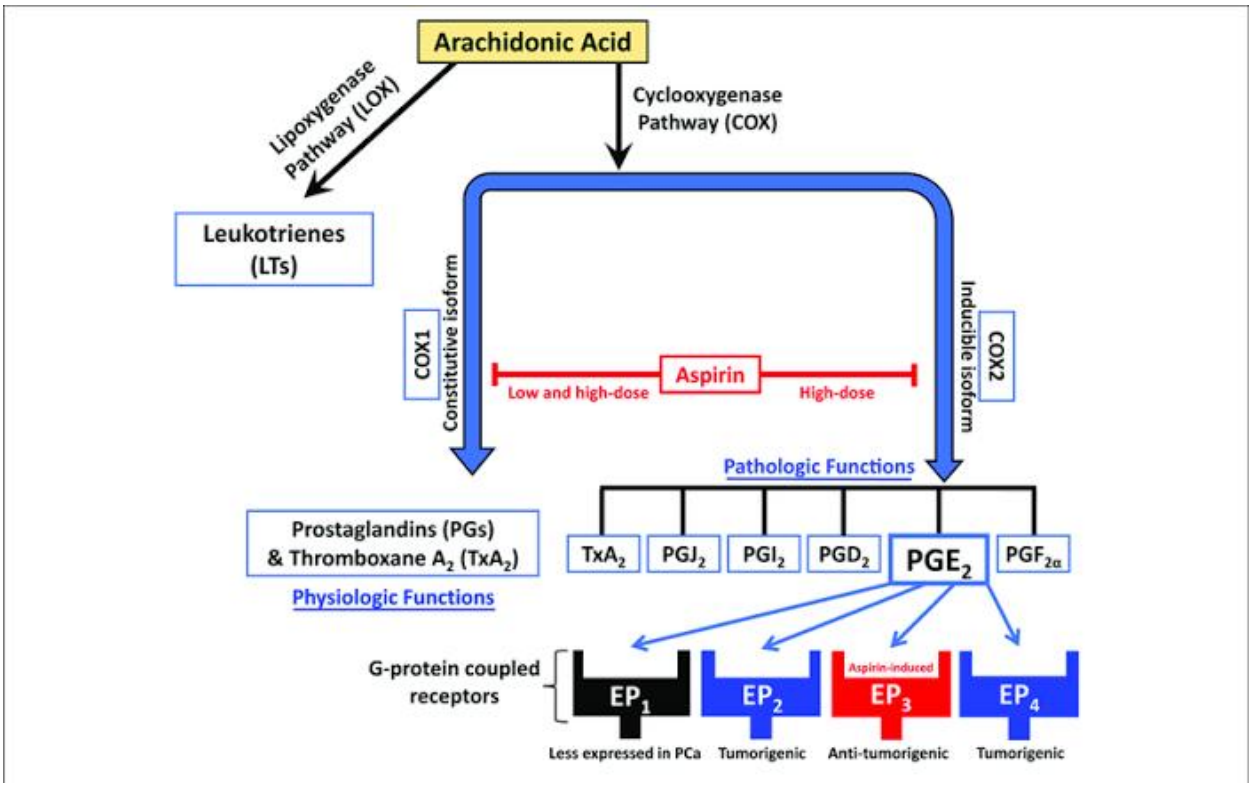

Fig 2.1: Diagram showing mechanism of action of Aspirin (NSAIDS), (Dhaghat *et al*., 2007)

Mechanism of action

Aspirin inhibits COX activity by covalently modifying, through acetylation, serine residues in the active sites of the COX enzymes. Inhibition of COX-1 is achieved by acetylation of a serine residue (ser 530), causing a conformational change in the enzyme, which disables it from oxidizing arachidonic acid. Conversely, acetylation of a serine residue (Ser 516) in COX-2 still enables it to metabolize arachidonic acid to 15-R-hydroxyeicosatetraenoic acid (15-R-HETE). This molecule can be subsequently metabolized by lipooxygenases to 15-epi-Lipoxin A4 (15 epi-LXA4 or aspirin triggered lipoxin ATL), a potent neutrophil inhibitor with protean anti-inflammatory effects and which has also been demonstrated to have potent anti-inflammatory effects on neutrophils, as well as stimulating resolution of inflammation. (Wallace et al., 2006). ATL has also been shown to dose-dependently induce the expression of haem-oxygenase-1 (HO-1), a ubiquitous and crucial tissue protective enzyme with vasodilative, anti-inflammatory and anti-oxidant properties, which may contribute to the impairment of leucocyte influx during the resolution phase of inflammation (Nascimento *et al*., 2005). Aspirin increases plasma (Chang *et al*., 2004) and urinary (Fiorucci *et al*., 2002) 15 epi-LXA4 in healthy human volunteers.

The protective effects of ATL have been demonstrated in the rat stomach, whereby blocking its production by concurrent administration of a COX-2 inhibitor and aspirin led to significantly increased gastric damage. (Fiorucci *et al*., 2002) This is the mechanism now thought responsible for the abrogation of the gastrointestinal safety of COX-2 inhibitors when used in combination with aspirin. (Devchand and Wallace, 2005) Administration of LXA4 parenterally significantly reduced aspirin induced damage and an LXA4 receptor antagonist (ALX) significantly worsened aspirin induced damage similar to a COX-2 inhibitor. 15 epi-LXA4 is thought to act through eliciting nitric oxide synthesis (from eNOS and iNOS), which ultimately inhibits leucocyte endothelial interaction. (Paul-Clark *et al*., 2005) provided evidence for this by demonstrating that aspirin inhibited leucocyte-endothelial adherence similar to nitric oxide in wild type mice, but both aspirin and 15 epi-LXA4 had markedly reduced effects on leucocyte endothelial adherence in eNOS- and iNOS-deficient mice compared with wild type mice (Paul and Cooper, 2004).

2.9.6 ULCER INDEX

The ulcer index of the control group and the UI and protective ratio (PR) of the treated groups were calculated using the following relations UI = (A*B)/100, where A= degree of ulceration, B= percentage of groups ulcerated, and PR = [(UIu_UIp) / UIu]*100, where UIu = ulcer index of ulcerated groups and UIp = ulcer idex of the protected groups. Degree of ulceration (DU) was calculated using the relation DU = (total ulcer score / number of ulcerated animals), (Ezike et Al., 2009).

2.9.7 ULCER SCORE

In 2013, (Tappuni et al) introduced the Ulcer Severity Score (USS) because of the lack of standardized assessment methods for aphthous stomatitis. [79] The USS incorporates six ulcer characteristics: number, size, duration, ulcer-free period, site, and pain. This scoring template may be of value to future studies assessing treatment efficacy, although at present it is not widely used.

2.9.8 TOTAL AND FREE ACIDITY

Total and free acid values are often measured and used to control phosphate systems, which are acidic by design (to initiate the phosphate reaction). The first titration would be for free acid. This would involve taking a bath sample and titrating it with a known standard such as sodium hydroxide (probably 0.1N or 1.0 N). An indicator is added to the solution that changes color when the titration reaches a certain pH. A common indicator for this would be bromphenol blue which changes from yellow to blue when passing through the pH range around 3.5-4.0. The free acid value is used to tell you how much acid is available to initiate the phosphate reaction and exists in its original “active” state. Too high a value may indicate that the bath would have difficulty initiating the phosphate reaction. The corrective action would be to add phosphoric acid to bring it in line with the supplier’s recommendations. Too low a value could indicate too much phosphoric acid in the system which will not “build” a coating since it would tend to strip it as quickly as it forms. The supplier may have a chemical to adjust for this (possibly mono- or disodium phosphate), or may just tell you to run some scrap parts or steel wool through the

system to use some of the available acid. This value will tend to rise and fall around some centerpoint that you will control around (i.e., five plus/minus 1 point).

2.9.9 THE PH OF THE GASTRIC JUICE

Gastric acid, gastric juice, or stomach acid, is a digestive fluid formed within the stomach lining. With a pH between 1 and 3, gastric acid plays a key role in digestion of proteins by activating digestive enzymes, which together break down the long chains of amino acids of proteins. Gastric acid is regulated in feedback systems to increase production when needed, such as after a meal. Other cells in the stomach produce bicarbonate, a base, to buffer the fluid, ensuring a regulated pH. These cells also produce mucus – a viscous barrier to prevent gastric acid from damaging the stomach. The pancreas further produces large amounts of bicarbonate and secretes bicarbonate through the pancreatic duct to the duodenum to neutralize gastric acid passing into the digestive tract. The active components of gastric acid are protons and chloride. Often simplistically described as hydrochloric acid, these species are produced by parietal cells in the gastric glands in the stomach. The secretion is a complex and relatively energetically expensive process. Parietal cells contain an extensive secretory network (called canaliculi) from which the "hydrochloric acid" is secreted into the lumen of the stomach. The pH of gastric acid is 1.5 to 3.5 in the human stomach lumen, a level maintained by the proton pump H+/K+ ATPase.[1] The parietal cell releases bicarbonate into the bloodstream in the process, which causes a temporary rise of pH in the blood, known as an alkaline tide. The highly acidic environment in the stomach lumen degrades proteins (e.g., food). Peptide bonds, which comprise proteins, are labilized. The gastric chief cells of the stomach secrete enzymes for protein breakdown (inactive pepsinogen, and in infancy rennin). The low pH activates pepsinogen into the enzyme pepsin, which then aids digestion by breaking the amino acid bonds, a process called proteolysis. In addition, many microorganisms are inhibited or destroyed in an acidic environment, preventing infection or sickness.

CHAPTER THREE

3.0. MATERIALS AND METHOD

3.1.0. MATERIALS

3.1.1. PLANT MATERIAL

Roots of *Chasmanthera dependens* (family Menispermaceae) were collected Kwara State and was identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria. A voucher specimen was deposited at the Herbarium of the department with voucher number UBHc – 397.

3.1.2. EQUIPMENTS/ APPARATUS

Table 2.1 Showing equipment used and their manufacturers

EQUIPMENT/ APPARATUS	**MANUFACTURER**
Beakers (250, 500 and 1000ml)	Pyrex (England)
Conical flasks (1000ml)	Pyrex (England)
Measuring cylinder (100ml, 1000ml)	Pyrex (England)
Separating funnel	Technics (England)
Analytical weighing balance	Golden Mettler H-80 (USA)
Stirrer	
Water distiller	B-Bran Sc. Inst. Company (England)
Simple weighing balance	Adventure OHAUS AR 1530
Rotary evaporator	RE-52A Shanghai YaRong, (China)
Aluminium foil	
Universal containers	
Sample containers	

Bucket centrifuge machine	Shanghai Youring International Co. Ltd. (China)
Pulverizing machine	Bodga Machinery Technology Co. Ltd. (China)
Pestle and mortar	
pH meter	ITT Analytics x.
Micro pipettes	Hawach Scientific Co, Ltd. (China)
Petri dishes	
Dissecting set	
Card board papers	
Refrigerator	

3.1.3. REAGENTS AND CHEMICALS

Table 2.2 Showing kits/ Assay and their reagents.

KITS/ASSAY	**CHEMICALS (REAGENTS)**
TOTAL AND FREE ACIDITY	• 0.01 N NaOH • Phenolphthalein • Topfer's reagent

3.1.4 ANIMALS USED

- **Gastroprotective screening:**

  A total number of twenty-five (25) albino Wistar rats weighing between (120g-200g) were used in the gastroprotective screening. The rats were randomly divided into three (3) control groups (n=15) and two (2) test groups (n=10).

3.1.5. REFERENCE DRUG USED

Misoprostol (300mcg/tablet) was used as the reference drug.

3.2.0. METHODS

3.2.1. METHOD OF EXTRACTION

Up to 700grams of the pulverized plant material was put into a glass jar. About 1400ml of methanol solvent was added and shaken vigorously for some time and left for a period of 5 days. It was then filtered with Whatman filter paper (size 1) and filtrate was evaporated by rotary evaporator. The residue obtained was used as the crude extract.

The concentration of the extract (% yield) is calculated as follows:

$$\frac{\text{Weight of dried extract (g)}}{\text{Weight of plant material used (g)}} \times 100\ (\%)$$

Where:

- Weight of dried extract= 17.1307g
- Weight of plant material used= 700g

  % yield= $\frac{17.1207}{700} \times 100\% = 2.445$

Therefore, concentration of the extract= 2.45

3.2.2. EXPERIMENTAL DESIGN

- Control groups (n=15)
- Gastroprotective (test) groups (n=10). Classified as follows
  - **Normal control (n=5):** Administered only clean water and commercial feed.
  - **Negative control (n=5):** Aspirin (300mg/kg) body weight.

- **Positive control (n=5):** Misoprostol (20mg/kg) body weight for seven (7) days + Aspirin (300mg/kg) body weight.
- **Gastroprotective (n=5):** *Chasmanthera dependens* extract (250mg/kg) body weight for seven (7days) + Aspirin (300mg/kg) body weight.
- **Gastroprotective (n=5):** *Chasmanthera dependens* extract (500mg/kg) body weight + Aspirin (300mg/kg) body weight.

- Where n= number of rats per group.

3.2.3. ULCER INDUCTION

The animals were fasted for a period of 24hours in separate clean cages having netted floor with openings for easy deposit of droppings on the drawers. This is to avoid coprophagia (Mabrouk *et al.,* 2009). They were allowed free access to water *ad libitum.* 300mg/kg body weight dose of aspirin was used to induce ulcer. After four (4) hours, under light anaesthesia by chloroform, the animals were sacrificed, stomach removed, washed, opened on greater curvature and examined for ulceration.

ULCER INDEX AND SCORING

The degree of ulceration was expressed in terms of ulcer score. Scoring method of ulceration according to Kuchandy *et al.,* (1985) was followed:

- 0= Normal grey coloured stomach
- 0.5 = Pink to red coloured stomach
- 1= Spot ulcer
- 1.5 = Haemorrhagic streak
- 2= Number of ulcer less than 5
- 3= Number of ulcer more than or equal 5
- 4= Ulcer with bleeding
- 5= Perforation of gastric or duodenal wall

Ulcer index was calculated according to the method of Kulkarni *et al.,* (2002).

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

Where UI= ulcer index

U_N= average number of ulcers per animal

U_S= average number of severity score

U_P= percentage of animal with ulcer incidence.

3.2.9. ULCER TOLERANT/HEALING RATE

The ulcer tolerant/healing rate was calculated according to the method of Alaribe *et al.* (2014).

$$\text{UTR/UHR \%} = \frac{\text{UI of the treatment group}}{\text{UI of the untreated controlled group}} \times 100$$

3.3 TOTAL AND FREE ACIDITY

Gastric juice (1 ml) was taken in to a 100 ml conical flask, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01 N NaOH until all traces of red colour disappears and the colour of the solution turns yellowish orange (end point). The volume of alkali added was noted. This volume corresponds to free acidity.2-3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. The volume of alkali added was noted which corresponds to total acidity.

Acidity was calculated by using the formula;

$$\text{Acidity (mEq/litre)} = \frac{\text{Volume of NaOH x Normality of NaOH x 100}}{0.1}$$

CHAPTER FOUR

RESULT

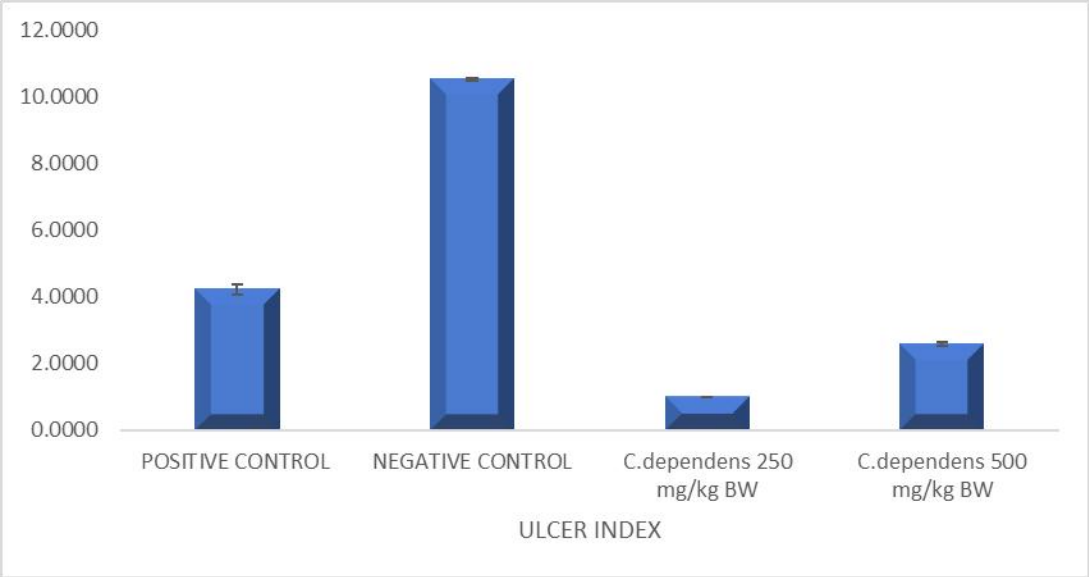

Fig 4.0: Effect of *Chasmanthera dependens* extract on the ulcer index in rats.

Group A: Positive control
Group B: Negative control
Group C: Normal control
Group D: *C.dependens* 250 mg/kg BW
Group E: *C.dependens* 500 mg/kg BW

Two doses of *C.dependens* (250mg/kg BW and 500mg/kg BW) given as treatment to aspirin induced ulcerated rats in groups D and C animals respectively, showed considerable inhibition of ulcer (see the fig above).

S/N	TREATMENT GROUPS(ASPIRIN)	Volume of gastric juice(ml)	pH	Acid output
1	POSITIVE CONTROL	0.77±0.02	3.18±0.78	5.08±1.2
2	NEGATIVE CONTROL	0.95±0.15	1.94±0.30	6.17±1.8
3	NORMAL CONTROL	0.20±0.06	3.70±0.13	3.83±0.85
4	*C.dependens* 250 mg/Kg BW	0.55±0.03	3.85±0.23	2.30±0.41
5	*C.dependens* 500 mg/Kg BW	0.60±0.82	3.46±0.56	3.60±0.37

Table 1.1 Table showing the various results of important parameters obtained during the experiment.

IMAGES SHOWING THE EFECTS OF C.DEPENDENS ON ASPIRIN INDUCED ULCERATED RATS

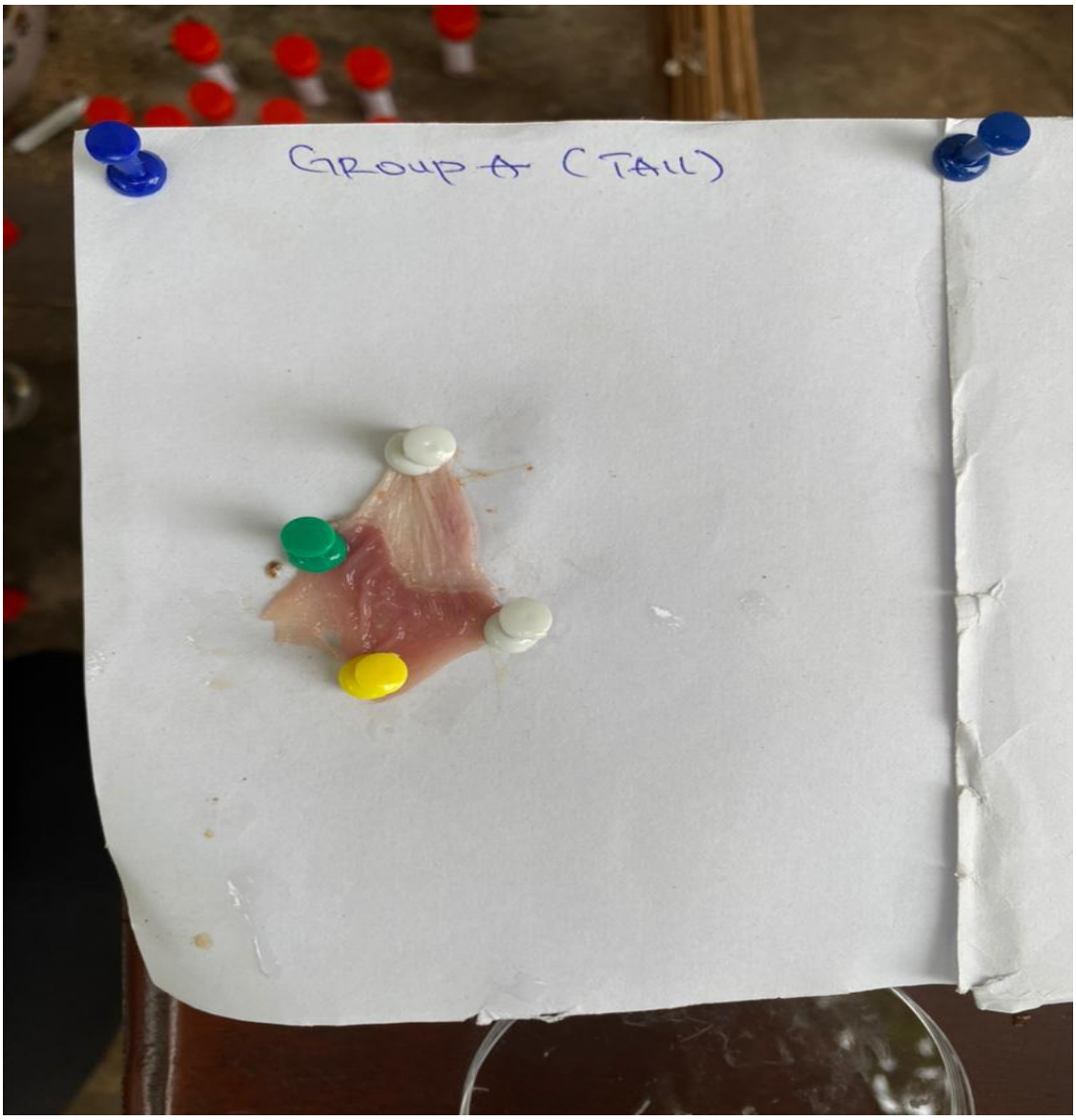

Fig 4.1; Group A (Normal control)

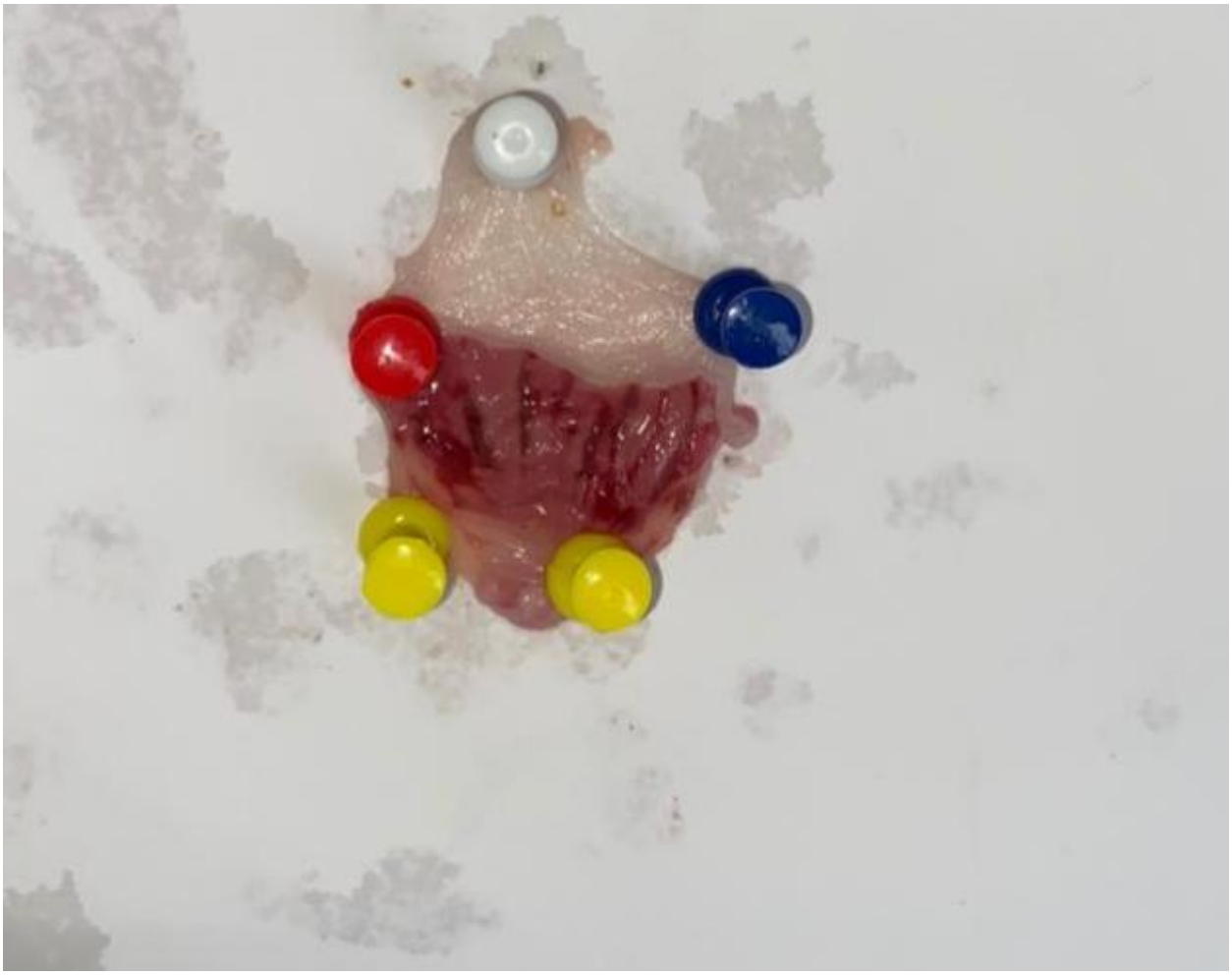

Fig 4.2; Group B (Negative control; Aspirin)

Fig 4.3; Group C (Positive control)

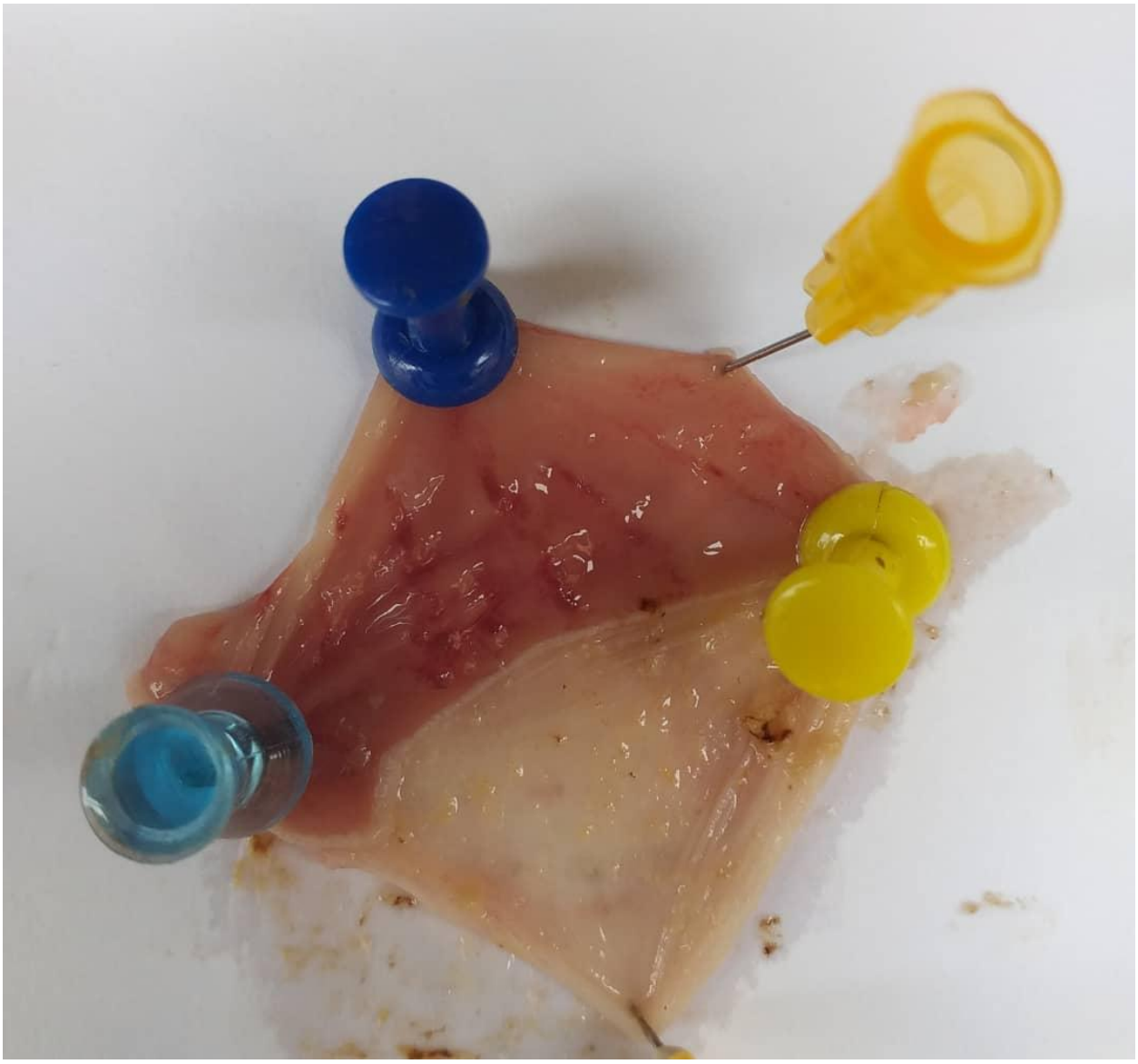

Fig 4.4; Group D [*C. Dependens* (250 mg/kg) + Aspirin (300mg/kg)]

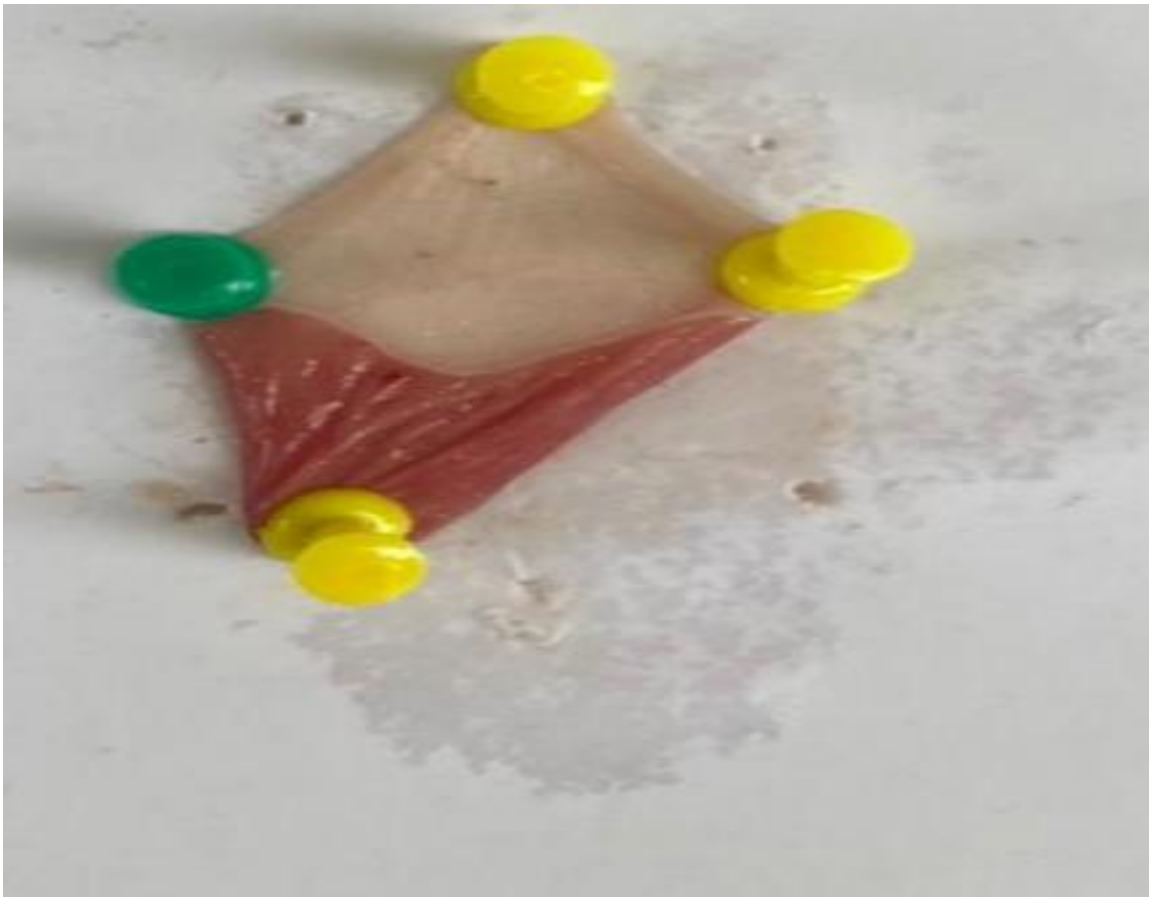

Fig 4.5; Group E [*C. Dependens* (500 mg/kg) + Aspirin (300 mg/kg)]

CHAPTER FIVE

5.0 DISCUSSION

Aspirin can induce ulcer in experimental animals. Aspirin commonly delayed gastric ulcer healing via a continuous increase in gastric acid secretion, a decrease of mucus content and bicarbonate secretion and increased generation of reactive oxygen species (ROS) leading to lipid peroxidation. The results of the present investigation revealed that administration of aspirin to rats caused significant ulceration in the glandular region of the rat stomach as evident by morphological assessment and a marked increase in ulcer index and acid output. However, treatments with methanol extract of *Chasmanthera dependens* and misoprostol decreased these ulcerogenic parameters. The decrease observed in the groups treated with methanol extract of *Chasmanthera dependens* roots can be attributed to the synergetic effects of the phytoconstituents present in the extract.

Gastric mucosa is normally well protected against aggressive and corrosive activities of gastric acid and pepsin by defensive cytoprotective factors such as mucus, bicarbonate, prostaglandin secretion and blood flow. The mucosal concentration of prostaglandin has been found to be directly related to the gastric mucus , bicarbonate secretion and blood flow (Komoike et Al., 2003). The observed increase in mean area of ulceration (gastric) in the negative control group animals treated with (300mg/kg body weight) of aspirin could be attributed to the reduction of mucosal prostalglandins biosynthesis accompanied by the inactivation of the cyclooxegenase system (COX) which mediates the synthesis of prostalglandins in the mucosa via the arachidonic acid pathwau (Maroney et al., 1988). However the different parts of the methanol extracts of C. dependens significantly ($p<0.05$) reduced the ulcer index in pretreated rats relative to negative control group. The extract siginificantly protected the gastric mucosa against injury suggesting cytoprotective activity and enhancement of mucosal defensive factors. The extracts produced a more potent activity than the standard reference drug misoprostol, the H2 receptor antagonist used as positive control group.

Anti-oxidant enzymes such as catalase, superoxide dismutase, gluthatione s-transferase and gluthatione are present in oxygen handling cells which are the first line of cellular defense against oxidative injury (Ologundu et al., 2008). Superoxide ions and H2O2 are decomposed before they interact to form more reactive radicals. Catalase is highly specific in its catalytic mode of action as it decreases the gastric mucosal damaging effect of NSAIDs.

The pH of the gastric juice plays a key role in the digestion of protein hence the need for the maintenance of and appropriate pH in the stomach, the pH of gastric acid is 1.5 to 3.5, the pH of the negative control group which is 1.94 indicates the presence of gastric ulcer, this is as a result of the aspirin ulcer induction and those of the other groups showed ph values between the normal range. Acid output values is a method for determining patients with gastric ulcer, acid outputs in patients with gastric ulcer tend to have an average higher acid output and average higher volume of gastric juice. The negative control group shows a slightly higher acid output and volume of gastric juice indicating gastric ulcer. Rats pretreated with extracts of *Chasmanthera dependens* showed a deduction in the acid output and volume of gastric juice compare to rats treated with aspirin (negative control group).

CONCLUSION

In conclusion, results of this study showed that methanol can effectively extract the active constituents responsible for anti-ulcerogenic properties. This result has therefore justified the use of extracts of roots of *Chasmanthera dependens* in the traditional treatment of gastric ulcers in Nigeria.

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