

**EFFECTS OF *Parkia biglobosa* FERMENTED SEED EXTRACT IN MALE WISTAR
RATS AFTER ASPIRIN-INDUCED GASTROINTESTINAL TRACT INJURIES**

BY

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BMS1702069**



**DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
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UNIVERSITY OF BENIN, BENIN CITY**

SEPTEMBER 2023.

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THIS PROJECT IS SUBMITTED TO:

**THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC
MEDICAL SCIENCE, COLLEGE OF MEDICINE, UNIVERSITY OF BENIN, IN
PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF
BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE**

SUPERVISOR: DR E.B. ODIGIE

SEPTEMBER 2023

CERTIFICATION

I certify that this project work was carried out by ANOZIE, CHINWENDU AMARACHI of the Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, under my supervision.

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DATE

DR. B.I.G. ADEJUMO
Ag. Head of Department

DATE

EXTERNAL EXAMINER

DATE

DEDICATION

This study is dedicated to God almighty and my parents, Mr. & Mrs. Anozie.

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First, I want appreciate God almighty for giving me the grace and strength to put together this project work.

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ABSTRACT

Parkia biglobosa (African locust beans) is a perennial plant, its seed has been reported to have good organoleptic containing high levels of fat, crude protein, pure protein, carbohydrates and starch. The fermented seed of *P.biglobosa* has been reported to contain an increased level of nutrients and secondary metabolites such as protein, fat, carbohydrates, phenols, saponins, flavonoids as compared to the unfermented seed. In various studies *p.biglobosa* has been shown to have antiulcer, antioxidant, anti-inflammatory therapeutic properties. Aspirin is a salicylic acid and a nonsteroidal anti-inflammatory drug (NSAID). Aspirin is used to reduce fever and relieve mild to moderate pain. Aspirin has been reported to have cardiovascular protective effect as well as adverse effects such as stomach ulcers and gastrointestinal bleeding. The aim of this study is to investigate fermented seed extract of *Parkia biglobosa* for its gastroprotective effect on aspirin induced gastrointestinal injury. Thirty male wistar rats were used for this study, which was divided into six groups having five rats per group (n=5). Animals in group one served as the unexposed group, groups two were fed 1000mg/kg of extract, group three were fed 20mg/kg of aspirin, group four, five and six were fed the extract and aspirin at alternated time frame. Histological changes as well as stomach weight, initial and final body weight were analyzed. Statistical software SPSS version 20 was used to analyze the data obtained. The statistical results showed no significant difference in increased stomach and body weight at p value <0.05. In the histological analysis of the oesophagus, stomach, small intestine and large intestine, group one and two were normal, group three had inflammation as a result of the aspirin administered. In group four the various parts analyzed were normal which showed the counter effect of *p.biglobosa*. Group five and six had various parts still inflamed which could be as a result of increased dosage or the duration of administration. In conclusion, it may be inferred that *Parkia biglobosa* has an effect, which is a counteracting effect on aspirin against gastrointestinal tract injury in male wistar rat.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Parkia biglobosa of the family Fabaceae and subfamily Mimosoideae, is a perennial legume tree popularly referred to as the African locust bean tree, monkey cutlass tree, or fern tree (Musara *et al.*, 2020). *Parkia biglobosa* is a vascular dicotyledonous angiosperm classified as spermatophytes. The African locust beans plant is a woody perennial deciduous tree that can grow as high as 30 meters. The fruit of *Parkia biglobosa* is a linear-oblong pod that matures from peach to dark brown color and is about 400 mm in length and 20 mm wide. It can contain up to 20 seeds that are enclosed in a yellow pericarp. The average seed weighs 0.26 g, is brown, smooth, and enormous. It has a firm, thick testa, and huge cotyledons that make up around 70% of its mass. The seeds is protected from the savanna's natural elements, including intense heat, low rainfall, drought, and ruminants' and primates' digestive secretions by the hard testa (Musara *et al.*, 2020).

Medicinal plants have greatly aided primary health care around the world. Plants have been utilized as food, medicine, and disease prevention for thousands of years (Silva *et al.*, 2010; Van wyk and Wink, 2018). Over the centuries, the knowledge of their healing properties has been passed down within and among human communities (Caballero-Serrano *et al.*, 2019; Soldati *et al.*, 2015). Their biological properties originates from the bioactive metabolite from the medicinal plants. Furthermore, as compared to conventional medication, medicinal plants are recognized to be active and toxicologically safe (Muhammed *et al.*, 2021). *P. biglobosa* has a great commercial value as a food and therapeutic agent. The traditional fermentation excellent food condiment is the most popular way African locust beans are consumed. To enhance the dietary quality and

sensorial acceptability of leguminous seeds. It has been reported by several researchers that processing technique heighten the dietary quality and also to dampens or eliminate the antinutrient in them (Esenwah and Ikenebomeh 2008).

P.biglobosa is an important tree species that produces non-timber forest products. It is a staple, healing food, and a source of riches. The fruit pods' pulp is high in sucrose, and the seeds are high in carbohydrates, proteins, and lipids, making them a significant source of energy. *P. biglobosa* is considered to be the fifth most significant of the thirty-one woody medicinal plants utilized in traditional Benin medicine (Builders et al., 2019). It is ranked fourth out of eighteen woody plants that should be preserved as priority food sources. The species work with crops to improve the physico-chemical composition of the soil, enhancing crop yields in the process (Builders *et al.*, 2019).

The gastrointestinal (GI) tract alongside other organs such as the liver, pancreas, and gallbladder constitute the digestive system. The GI tract is composed of a several hollow organs that link each other from the mouth to the pharynx (throat), esophagus, stomach, small intestine, large intestine, rectum, and anus. The digestive system is specifically modelled to carry out its physiological function of converting food into the nutrients and energy needed to exist. Thereafter, it efficiently compresses the solid waste, for disposal (Cleveland clinic, 2021).

Aspirin is a nonsteroidal anti-inflammatory drug (NSAID). It was the first drug of its kind to be found. Salicylate, a compound present in plants like the myrtle and willow tree, is a constituent of aspirin. It was first used approximately four thousand years ago (Tawfeek *et al*, 2021). The mechanism of action is by reducing the levels of chemicals in the body that causes inflammation, fever, and discomfort. Aspirin is an analgesic that also ameliorate fever and inflammation. It is

sometimes used for the treatment or prevention of heart attacks, strokes, and chest pain (Sinha, 2018).

1.2 AIM

The purpose of this research is to investigate *Parkia biglobosa* fermented seed extract for its gastroprotective, reversal, or counter effect after aspirin-induced gastrointestinal tract injury.

1.3 OBJECTIVE OF STUDY

1. To examine the effects of *P. biglobosa* fermented seed extract on aspirin-induced toxicity in the gastrointestinal tract.
2. To determine the potentiality of *P. biglobosa* fermented seed extract as a gastroprotective agents against aspirin overdose
3. To determine the efficiency of *P. biglobosa* fermented seed extract to reverse the effect of aspirin-induced gastrointestinal tract injury.
4. To observe the capability of *p. biglobosa* fermented seed extract to counter the effect of aspirin overdose on gastrointestinal tract.

1.4 RESEARCH QUESTION

1. Is it possible to examine the effect of *P. biglobosa* fermented seed extract on aspirin-induced toxicity in the gastrointestinal tract?
2. Can *P. biglobosa* fermented seed extract serve as a gastroprotective agent against aspirin toxicity?
3. Will *P. biglobosa* fermented seed extract be able to reverse the effect of aspirin-induced gastrointestinal tract injury?

4. Is it possible for *P. biglobosa* fermented seed extract to counter the effect of aspirin overdose on gastrointestinal tract?

1.5 HYPOTHESIS

The null and alternative hypothesis which has tested in this study includes;

Ho1; null; The extract of *P. biglobosa* fermented seed has no effects on aspirin-induced toxicity of the gastrointestinal tract.

Alt; The extract of *P. biglobosa* fermented seed has positive effects on aspirin-induced toxicity of the gastrointestinal tract.

Ho2; null; *P. biglobosa* fermented seed extract cannot prevent gastrointestinal tract injury resulting from aspirin overdose.

Alt; *P. biglobosa* fermented seed extract can prevent gastrointestinal tract injury resulting from aspirin overdose.

Ho3; null; *P. biglobosa* fermented seed extract does not have the ability to reverse the effects of aspirin-induced gastrointestinal tract injury.

Alt; *P. biglobosa* fermented seed extract has the ability to reverse the effects of aspirin-induced gastrointestinal tract injury.

Ho4; null; *P. biglobosa* fermented seed extract cannot counter the effects of aspirin overdose on the gastrointestinal tract.

Alt; *P. biglobosa* fermented seed extract can counter the effects of aspirin overdose on the gastrointestinal tract.

1.6 STATEMENT OF PROBLEM

Aspirin, also known as acetylsalicylic acid, is frequently used in therapeutic analgesic, antiplatelet, and antipyretic therapy. Aspirin possesses a wide range of negative results on the gastrointestinal (GI) tract, from uncomfortable GI symptoms without lesions of the stomach mucosa to ulcers, bleeding, and even fatalities (Zhuoya et al., 2020). The best option for treating aspirin-induced gastrointestinal damage may be omeprazole plus rebamipide (Wan-tong et al., 2021). However, limited studies have been carried out on the protective, reversal or counter effect of fermented seed extract on *Parkia biglobosa* after aspirin-induced gastrointestinal tract injury.

1.7 JUSTIFICATION OF STUDY

The gastrointestinal tract plays a crucial function in controlling energy balance because it serves as the main point of contact between the body and ingested nutrients. In order to keep the body's energy balance, the brain receives signals from the gut that inform it about the nutrients that are coming in. This information prompts changes in eating habits and energy use (Monteiro and Batterham, 2017). Ascribed to the increased use of nonsteroidal anti-inflammatory medicines (NSAIDs) such as aspirin, which are known to produce ulcers, ulcers are recognized as the most frequent reason for upper gastrointestinal (GI) bleeding hospitalizations and continue to be a significant clinical concern (Cryer and Mahaffey, 2014). The importance of the gastrointestinal tract cannot be overemphasized, therefore maintaining its integrity is a priority. *P. biglobosa* fermented seed extract might aid in the protection, reversal, or counter-effect of aspirin-induced gastrointestinal tract injury, which is the bases for this study.

1.8 SIGNIFICANCE OF STUDY

Several guidelines advise individuals with past cardiovascular disease or numerous risk factors to take aspirin on a long-term basis to prevent cardiovascular events. Thus far aspirin is connected to an elevated risk of severe gastrointestinal bleeding. According to a recent meta-analysis, aspirin users experience close to a dual increased risk of gastrointestinal bleeding than those who take a placebo (Huang et al., 2011).

A positive outcome of this study will be beneficial to medical professionals to enable them to oversee the well-being of the patient, and prevent, reverse or counter the effect of aspirin-induced gastrointestinal tract injury for patients who are on long-term use of aspirin for cardiovascular disease and ameliorate the effect of aspirin overdose by individuals.

CHAPTER TWO

LITERATURE REVIEW

2.1 AFRICAN LOCUST BEANS (*P. bilobosa*)

Medicinal plants have been utilized in healthcare since the ancient time. Comprehensive research has been carried out to confirm their efficacy, and some of the results have triggered the development of plant-based medications (Abayomi *et.al*, 2013). Within human groups, the empirical knowledge of their positive impacts has been passed down through the ages (Mariangela, 2021). Due to its beneficial physicochemical qualities, the use of plant-derived meals in the prevention, treatment, and management of metabolic illnesses has grown in popularity (Bolajoko *et al*, 2016).

P. biglobosa is widely distributed in the natural savanna zones of the Sudan and Guinea. The range stretches from Senegal on Africa's western coast all the way to Sudan. *P. biglobosa* is present in 19 African nations they include; Senegal, Togo, Gambia, Guinea Bissau, Uganda , Guinea, Sierra Leone, Mali, Côte d'Ivoire, Burkina Faso, Benin, Niger, Nigeria, Cameroon, Central African Republic, Zaire, Ghana, Sudan, and Chad (Builders, 2014).

African locust bean, or *P. biglobosa*, is a plant that is widely utilized in West Africa for food, medicinal, and building materials. In tropical Africa, it was widely recommended in traditional medicine for a variety of health benefits (Komolafe *et al*, 2017). Food is the most important commodity produced by *p.biglobosa* tree, and it is essential since it matures and is available only during certain seasons. It supports household food reliability, livelihood, and poverty depletion during times of food scarcity and drought. In the savannah zones of sub-Saharan Africa, fermented *P. biglobosa* seeds are used to season local soups. The fermented seeds, also known as

"Iru" are processed and marketed in Nigeria as fresh or dried spices or cooking condiments (Musara et al., 2020). It is also known as Ogiri in Igbo, and Dawadawa in Hausa territory of Nigeria (Oloyede and Akintunde 2019). They can be used as ingredients to make different stews, soups, and sauces that are served with cereal. They can also be fermented into an alcoholic beverage or pressed into cakes. The roasted seeds are used to make "Sudan coffee" or "café nègre," a type of coffee replacement (Musara et al., 2020).

The seeds have good organoleptic qualities and comprise 35% proteins, 29% fats, 16% carbohydrates, and calcium. There is also a non-toxic oil with a diverse composition. Arachidic acid is said to be the largest prevalent fatty acid, followed by behenic, stearic, palmitic, and linoleic acids, according to some authors. The nutritional contents of *P. biglobosa* seeds were proximally analyzed, and the results showed that they contained significant amounts of starch, fat, pure protein, crude protein, carbohydrates and total soluble sugar (Builders, 2014).

2.2 FERMENTED *P. biglobosa* SEED

Fermentation is the process through which bacteria use extracellular interactions to alter food, typically legumes (and cereals), for preservation or to generate desirable, sensory properties such as flavors and texture (Adebo *et al*, 2020). It has been observed that fermenting *P. biglobosa* seeds into dawadawa enriches the fermented product with proteins, amino acids, vitamins, fatty acids, improved antioxidant activity, and eliminates or reduces antinutrients (Oboh *et al*, 2008; Ndukwe *et al*, 2017). The fermented locust bean seed helps with digestion, promotes good vision, and lowers cholesterol levels. Blood sugar levels are lowered and bacterial illnesses are treated with water and alcohol extract fermented locust bean. One of the ancient food preservation techniques in practice today and the world's oldest preservation technology is fermentation.

Although raw African locust beans are nutritionally lacking and unpleasant to eat, their morphological, chemical, and nutritional qualities alter instantly after fermentation (Oloyede and Akintunde 2019). In fermented locust bean, phytochemical analysis revealed 1.08mg/g oxalate, 8.16mg/g flavonoid, 3.18mg/g saponin, 16.48mg/g phytate, 7.08mg/g phenol, and 5.39mg/g tannin (Egbebi *et al*, 2016).

According to reports, the microorganisms responsible for the fermentation of African locust beans include *Staphylococcus spp.* and *subtilis*. During the fermentation process, they hydrolyze proteins into amino acids and ammonia, which results in an increase in pH. The majority of these fermentations occur naturally, entail contact with the proper inocula, and are facilitated by tropical temperatures. Increased nutritional content, toxin elimination, improved texture, taste, and flavor are just a few of the advantages of fermentation (Oloyede and Akintunde 2019).

2.2.1 PROCEDURE FOR FERMENTATION

The following are steps involved in the fermentation process of *P. biglobosa* as recorded by Osuntokun *et al* 2020

1. In order to loosen the firmly attached seed coats, boil raw *P. biglobosa* beans with pressure-cooker for 6 hours.
2. Further soak in the boiling water for an additional 6 hours.
3. Drained off excess water, lightly pound the seed with a big wooden mortar and pestle to remove the seed coat.
4. Rub the cotyledons between the palms of the hands before thoroughly rinsing with water.
5. Once again pressure-cook the cotyledons for 3 hours.
6. Drained out the hot boiling water, pack the cotyledons securely in a sack, place in a bowl, and cover with wooden trays to keep the environment warm.

7. This process takes 5 days.



Figure 2.1: (a) Unfermented seeds of *parkia biglobosa* (b) fermented seeds of *parkia biglobosa* (Osuntokun et al., 2020).

2.3 MEDICAL USES OF *P. biglobosa*

The medical uses of *P. biglobosa* includes the following;

i. Anti-inflammatory Effect

Almost all clinical manifestations entail an inflammatory reaction. Therefore, the anti-inflammatory properties of several plant extracts may be advantageous. Several models of inflammation have been used to test the anti-inflammatory effects of *P. biglobosa* seeds (Kouadio *et al*, 2000). *P. biglobosa* is thought to exert its protective benefits by inhibiting the lipoxygenase and cyclooxygenase pathways (Nwaehujor *et al*, 2010). This inhibits the expression of pro-inflammatory cytokines and activate the production of anti-inflammatory cytokines (Silva *et al*, 2013) while also increasing membrane stability (Ukwuani *et al*, 2015). The bioactive components of plants clearly reduce inflammation by controlling inflammatory and mitogen-activated protein kinase (MAPK) signaling pathways, which may lower the production of inflammatory mediators like cytokines (Mohammed *et al*, 2021).

ii. Anticancer Activity

One of the diseases that causes millions of death globally is cancer (Mohammed *et al*, 2021). An *in vitro* study on human cancer cell lines revealed that *P. biglobosa* methanol extracts have varying measures of antiproliferative activity on T-549 and BT-20 (prostate cancer), PC-3 (acute T cell leukemia Jurka), and SW-480 (colon cancer) at concentrations of 20 and 200 g/ml. The results also showed that *P. biglobosa* has stronger cytotoxic effect on all cancer cell lines tested (Fadeyi *et al*, 2013).

iii. Anti-anemic Effect

P.biglobosa fermented seeds are an abundant source of important minerals such as calcium, iron, thiamine, and phosphorus (Campbell *et al*, 1980) which are required to prevent anemia due to either iron or non-iron deficient. As a result, *P. biglobosa*'s antianemic potential may be due to its nutritional makeup. *P.biglobosa* fermented seeds, when combined with more fermented items, were found to be advantageous in the treatment of anemia, increasing red blood cells, packed cell volume, hemoglobin and white blood cells (Ijarotimi *et al*, 2012).

iv. Antioxidant Activity

Polyphenolic chemicals found in plant diets have been linked to antioxidant activity as a result of their potential to operate as a reducing agent and hydrogen donor. *P. biglobosa* seeds, both fermented and unfermented, have been found to have significant phenolic content (Badu *et al*, 2012, Oboh *et al*, 2008) .

v. Antihypertensive Activity

Parkia biglobosa seeds have been shown to have antihypertensive activity in both animals and humans. Only one clinical trial was undertaken, and it found that individuals with hypertension who consumed fermented seeds of *P. biglobosa* had reduced blood pressure, blood glucose, and heart rate, as well as a higher magnesium level and an improved lipid profile as compared to the non-consumption group (Ognatan *et al*, 2011). In a rat model, 1.9 mg/mL of *P. biglobosa* seed extract decreases arterial blood pressure, presumably as a result of its potential to slow the heart rate (Kodjo *et al*, 2006) and induce vascular relaxation (Ouédraogo *et al*, 2012).

vi. Antidiabetic Activity

Alloxan-induced diabetic rats when evaluated after four weeks, administration of *P. biglobosa* methanol and aqueous extracts of fermented seeds orally demonstrated varying levels of hypoglycemic effects on fasting plasma glucose (Odetola *et al*, 2006; Sule *et al*, 2015). In alloxan-induced diabetic mice, oral treatment of *Parkia biglobosa* seeds methanol extract (1 g/kg body weight) reduced blood glucose levels by 44.1% at 8 hours in contrast with glibenclamide (37.9%). The chloroform fraction had the highest glucose-lowering impact (65.7%), whereas the n-hexane fraction had the least (4.7%) (Ezema *et al*, 2016). A similar underlying mechanism of plant species hypoglycemic effect is proposed, which is through an enhancement in pancreatic islet activities to express insulin while eliminating insulin resistance (Ibrahim *et al*, 2016).

2.4 PHYTOCHEMICAL

Plants are abundant in chemical components that have therapeutic qualities that support and enhance human health. These chemical components include secondary metabolites and other phytochemical compounds. The bioactive plant components found in secondary metabolites are also included in many medications as active ingredients. Terpenes, alkaloids, flavonoids, and phenolic chemicals are the most crucial of these plant bioactive components. Others include tannins, saponins, glycosides and steroids (Osuntokun *et al*, 2020).

i. ALKALOIDS

Alkaloids are used for their analgesic, antispasmodic, and antibacterial actions. They are also known to have a muscle relaxant activity.

ii. SAPONINS

The seeds' saponin content makes them an excellent anti-inflammatory agent. Red blood cells can precipitate and coagulate as a result of saponins. The ability to create foams in aqueous solutions, hemolytic activity, bitterness and cholesterol-binding abilities are some of the traits of saponins (Rita *et al*, 2015). In humans, saponins might have the ability to combat cancer and infection (Adelani *et al*, 2015).

iii. TANNINS

Tannins have an astringent flavor and aid in the healing of wounds and inflamed mucous membranes (Osuntokun *et al*, 2020). Tannins reduce bacterial growth by inhibiting vital enzymes involved in microbial metabolism. The seeds may therefore function as a powerful antibacterial medication (Pradeepa *et al*, 2016).

iv. FLAVONOIDS

Flavonoids in plants are a diverse group of physiologically active substances that have long been employed in traditional medicine for their antioxidant and antiproliferative properties, particularly in the treatment of allergy illnesses and chronic inflammatory, coronary artery disease and breast cancer (Ochwang'I *et al*, 2016).

v. TERPENOIDS

Terpenoids are antifungal and antibacterial agents which is associated to their membrane disruption activity and inhibitory potential on bacterial cell or fungus (Tawheed and Monika, 2014).

vi. GLYCOSIDES

Glycosides can be utilized in the remedy of congestive heart failure and are advantageous in lowering inflammation and guarding against endotoxemia (Tawheed and Monik, 2014).

vii. STERIODS

Steroids is present in minimal proportion. It has been demonstrated that steroid compounds in plants have analgesic effects and are involved in central nervous system functions (Ahmed and Mohammad, 2014).

2.5 GASTROINTESTINAL TRACT

The gastrointestinal tract is made up of several hollow organs from the mouth to the anus connected by a extended, twisted tube. The Gastrointestinal tract is made up of hollow organs including the stomach, small intestine, large intestine, and anus. The digestive system's solid organs are the liver, pancreas, and gallbladder. The small intestine is made up of three sections, the duodenum, ileum and jejunum. The first section is the duodenum, the jejunum at the middle and the ileum is at the end. The cecum, colon, rectum, and appendix make up the large intestine. A finger-shaped pouch connected to the cecum is the appendix. The cecum is the beginning of the large intestine and the colon follows. The large intestine empties the waste into the rectum and the waste product is released through the anus (National Institute of Diabetes and Digestive and Kidney Diseases, 2017).

Functions of the Human Digestive System

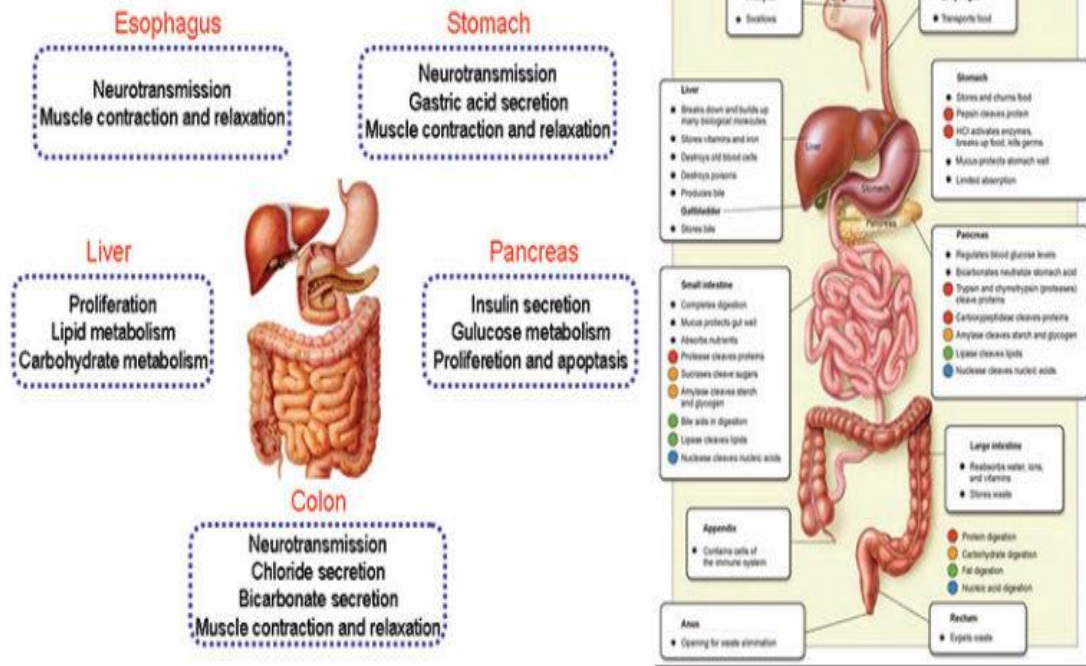


Figure 2.2: Architecture of the digestive system. The mouth, pharynx, esophagus, stomach, small intestine, large intestine, rectum, and anus are part of the digestive system (Sagar, 2019).

2.5.1 HISTOLOGY OF THE GASTROINTESTINAL TRACT

The alimentary canal has a common pattern of histological organization such that the lumen is lined by a mucosa, which comprises of epithelium, a loose, cellular connective tissue that enclose glands, the lamina propria, and the muscularis mucosae. The mucosa is surrounded by a denser connective tissue known as the submucosa, which have glands only in the esophagus and duodenum. The muscularis externa surrounds the submucosa, which is then covered by a serosa or an adventitia. Variations in these layers' components permit regional, structural and functional specialization of the alimentary canal (Leslie *et al*, 2011).

OESOPHAGUS

Stratified squamous nonkeratinized epithelium lines the oesophagus. The lamina propria consists of mucus-secreting oesophageal cardiac glands, and the submucosa has mucus-secreting oesophageal glands proper. The muscularis mucosae differs in thickness and is made up of a single longitudinal layer of smooth muscle. The upper third of the muscularis externa is comprises of only skeletal muscle; the middle third consist of a combination of smooth and skeletal muscle; and the lower third is composed only of smooth muscle. The oesophagus conveys a bolus of food from the pharynx into the stomach by peristaltic activity of the muscularis externa. Two physiological sphincters (the pharyngoesophageal and the gastroesophageal) in the muscularis externa ensure that the bolus is transported in one direction only, toward the stomach (Leslie *et al*, 2011).

STOMACH

The stomach acidifies and transforms the bolus into a thick, viscous fluid called as chyme. It also produces digestive enzymes and hormones. The stomach displays longitudinal folds of the

mucosa and submucosa (called rugae), which disappear in the distended stomach. It is composed of numerous gastric pits (foveolae), which are shallowest in the cardia and deepest in the pylorus. The simple columnar epithelium of the gastric mucosa is made up of mucinogen-producing surface lining cells (Leslie et al, 2011).

The lamina propria is a loose connective tissue enclosing smooth muscle cells, lymphocytes, plasma cells, mast cells, and fibroblasts. It comprises of gastric glands. The muscularis mucosae is composed of a poorly outlined inner circular layer, an outer longitudinal layer, and occasionally an outermost circular layer of smooth muscle. Gastric submucosa is composed of dense, irregular collagenous connective tissue. It made up of fibroblasts, mast cells, and lymphoid elements embedded in the connective tissue. The gastric submucosa houses Meissner (submucosal) plexus, also have arterial and venous plexuses that respectively supply and drain the vessels of the mucosa (Leslie et al, 2011). Gastric muscularis externa is have three layers of smooth muscle, an incomplete inner oblique layer, a thick middle circular layer that forms the pyloric sphincter, and an outer longitudinal layer. Auerbach myenteric plexus is found between the middle circular and outer longitudinal smooth muscle layers, mixing of gastric contents and emptying of the stomach are the function. The gastric muscularis externa is affected by diverse characteristics of the chyme (e.g., lipid content, viscosity, osmolality, caloric density, and pH), which influence the emptying rate of the stomach and the rate of muscle contraction. External surface of the stomach is covered by serous (Leslie et al, 2011).

SMALL INTESTINE

The small intestine is approximately 7 m long and has three regions, the duodenum which is at the proximal portion, jejunum at the middle, and ileum at the distal. The small intestine secretes

a number of hormones, it completes the digestion of food materials and absorbs the resulting metabolites. The luminal surface of the small intestine has plicae circulares, intestinal villi, and microvilli, which collectively elevates the luminal surface area by a factor of 400 to 600 (Leslie *et al*, 2011). The mucosa of the small intestine is simple columnar epithelium. It is made up of goblet cells, surface absorptive cells, and some DNES cells. Lamina propria occupies the cores of the villi and the interstices between the numerous glands (crypts) of Lieberkühn (Leslie *et al*, 2011). The lamina propria consists of loose connective tissue with lymphoid cells, fibroblasts, mast cells, smooth muscle cells, nerve endings, and lymphoid nodules, It also contains lacteals (blind-ended lymphatic vessels) and capillary loops. The muscularis mucosae is composed of an inner circular and an outer longitudinal layer of smooth muscle (Leslie *et al*, 2011).

Submucosa of the small intestine consists of fibroelastic connective tissue containing blood and lymphatic vessels, nerve fibers, and Meissner plexus, also encloses Brunner glands, which are present only in the duodenum. Brunner's glands produce an alkaline fluid and urogastrone (Leslie *et al*, 2011). The alkaline fluid protects the duodenal epithelium from the acidic chyme where as the urogastrone is a polypeptide hormone (human epidermal growth factor) that increases epithelial cell division and impedes gastric HCl production (Leslie *et al*, 2011). The muscularis externa of the small intestine is have two layers of smooth muscle, an inner circular and an outer longitudinal layer. The inner layer participates in the formation of the ileocecal sphincter. Auerbach (myenteric) plexus is encased between the two layers. In external layer of the small intestine serosa covers all of the jejunum and ileum and part of the duodenum and adventitia covers the remainder of the duodenum (Leslie *et al*, 2011).

LARGE INTESTINE

The large intestine comprises the cecum, colon (ascending, transverse, descending, and sigmoid), rectum, anal canal, and appendix. The large intestine have some digestive enzymes received from the small intestine. The large intestine holds bacteria that produce vitamin B12 and vitamin K. Vitamin B12 is necessary for hemopoiesis and the vitamin K for coagulation (Leslie *et al*, 2011). The large intestine produces ample mucus, which lubricates its lining and aids the passage and elimination of feces. The large intestine functions essentially in the absorption of electrolytes, fluids, and gases. Dead bacteria and indigestible residue of the ingested material are compacted into feces. The mucosa of the cecum and colon lacks villi and possesses no specialized folds (Leslie *et al*, 2011). The mucosa of the cecum and colon is simple columnar epithelium with numerous goblet cells, surface absorptive cells, and occasional DNES cells. The lamina propria is analogous to that of the small intestine, having lymphoid nodules, blood and lymph vessels, and closely packed crypts of Lieberkühn, which do not have Paneth cells (Leslie *et al*, 2011). The muscularis mucosae consists of an inner circular and outer longitudinal layer of smooth muscle cells. The submucosa of the cecum and colon is composed of fibroelastic connective tissue, It contains blood and lymphatic vessels, nerves, and Meissner (submucosal) plexus. The muscularis externa of the cecum and colon is comprises of an inner circular and a modified outer longitudinal layer of smooth muscle (Leslie *et al*, 2011). The outer layer is gathered into three flat, longitudinal ribbons of smooth muscle that form the teniae coli. When continuously contracted, the teniae coli form sacculations of the wall called haustra coli. Auerbach (myenteric) plexus is encased between the two layers of smooth muscle. In the external layer of the cecum and colon, the adventitia covers the ascending and descending portions of the colon while the

serosa covers the cecum and the remainder of the colon. Fat-filled outpocketings of the serosa (appendices epiploicae) are characteristic of the transverse and sigmoid colon (Leslie *et al*, 2011).

2.5.2 GASTROINTESTINAL TRACT INJURY

The array of medications that can affect the gastrointestinal tract is astonishing, and drug toxicity in the gastrointestinal tract is a worldwide and important medical problem. Medication toxicity causes morbidity, mortality, and high medical expenditures even when limited to gastrointestinal tract and are likely underestimated. Drug-induced gastrointestinal tract injuries are quite diverse and can initiate numerous diseases that are not caused by drugs. Whether direct or indirect, a part of the gastrointestinal tract may be spared from drug toxicity or the entire gastrointestinal tract may be affected. Drug toxicity can have a wide range of effects, from quite common and generally benign pathologies (such relatively common and benign drug-induced diarrhea) at one end of the spectrum to catastrophic gastrointestinal tract hemorrhage or perforation at the other (Pusztaszeri *et al.*, 2007).

2.6 ASPIRIN

The most often prescribed substance in medicine is aspirin. According to a 2007 assessment by the Agency for Healthcare Research and Quality (AHRQ), about 20% of American adults admitted to using aspirin daily or every other day, and this percentage rose to nearly 50% in those 65 and older. The history of aspirin can be traced back to Hippocrates and Galen's time, when the willow tree's bark was well known for its analgesic and anti-inflammatory properties. Aspirin is also one of the oldest medicines still in use today (Ittaman *et al.*, 2014). In persons with cardiovascular disease or in those who have already experienced a heart attack or stroke, aspirin has been shown to be beneficial when administered daily to ameliorate the risk of heart

attack, clot-related strokes, and other blood flow issues. Aspirin is often recommended by doctors for these purposes (Center for Drug Evaluation and Research, 2019).

2.6.1 MECHANISM OF ACTION

The upper gastrointestinal (GI) tract injuries affecting mucosa which is caused by aspirin's negative effects are mediated by both direct and indirect mechanisms (Carl et al, 2017). The potential of aspirin (a weak acid) to penetrate the mucous layer that coats the gastric mucosa and enter the plasma membrane of gastric epithelial cells, whereupon the molecule dissociates, trapping hydrogen ions within the cells, brings about direct topical injury (Wolfe et al, 1999). The major mechanism for its harmful effects on the mucosa of the upper GI tract, however, is systemic, via inhibition of cyclooxygenase and resultant decrease in prostaglandin synthesis (Wolfe et al, 1999; Wallace, 2008). Prostaglandins are gastroprotective, and aspirin effects include reduced gastric epithelial release of bicarbonate and mucus, diminished gastric mucosal blood flow, and decreased epithelial cell proliferation (Wolfe et al, 1999; Wallace, 2008). As a result, the gastric mucosa becomes more susceptible to harm from exogenous and endogenous substances (for example, stomach acid and pepsin) and has a reduced ability to repair itself (Wolfe et al, 1999; Fanaroff and Roe, 2016). Moreover, the inhibition of platelet aggregation that promotes aspirin's antithrombotic activity predisposes to GI bleeding once mucosal damage occurs (Fanaroff and Roe, 2016). Clinically, the spectrum of aspirin-induced GI toxicity caused by these pathways includes superficial mucosal injury (subepithelial bleeding and erosions) and mucosal ulceration; however, this can eventually intensify to hemorrhage, perforation, or both, which can lead to death (Wolfe *et al*, 1999).

2.6.2 PHARMACOKINETICS

How well aspirin is absorbed from the gastrointestinal (GI) system is affected by the formulation state. In opposition to pills, it is quickly absorbed when ingested as a liquid mixture. Its hydrolysis produces salicylic acid, and there is a small therapeutic window for salicylic acid. If maintained within that certain range, it has the needed anti-inflammatory effects. At the level of the small intestine, aspirin absorption is pH sensitive, and the same pH range, small intestine absorption is higher than stomach absorption. Aspirin's intestinal absorption is larger than its gastric absorption at pH 3.5 or 6.5. The stomach cannot absorb aspirin at a pH of 6.5 (Arif et al., 2019).

2.6.3 ADVERSE EFFECTS OF ASPIRIN

Aspirin has undergone numerous metanalyses, which shows that it tends to increase the rates of bleeding and gastrointestinal pathology while also reducing the risk of significant detrimental cardiovascular events in individuals with diabetes without cardiovascular disease. Hypersensitivity, Reye syndrome, intracerebral hemorrhage, gastritis and gastrointestinal bleeding are among the side effects of aspirin (Arif et al., 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 LOCATION AND DURATION OF STUDY

This research was conducted at the Department of Medical Laboratory Science, (Histopathology Unit Laboratory), School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City: Edo State, Nigeria. The study started on the 26th of January, 2023, and lasted till the 9th of April, 2023.

3.2 REAGENTS AND CHEMICALS

Haematoxylin dye, eosin dye, 1% acid-alcohol, distrene plasticizer, 10% neutral buffered formalin, xylene and normal saline are some of the ingredients (All reagents were distilled before use)

3.3 EQUIPMENT AND APPARATUS

Dissecting materials: Dissecting set, Dissecting board, Cotton Wool, Husks, Gauze. Glassware (Pyrex): Measuring Cylinder, cover slip, Conical Flask, Slides, Universal Containers, 5ml syringes. Tissue Processing Materials: Automatic Tissue Processor (Hestion -ATP7000 tissue processor-Germany), Binocular Soxhlet extractor, Analytical weighing balance, plastic cages, Microscope (Olympus England), digital electronic balance (Gilbertini, Italy; sensitivity = 0.001g), Embedding Machine (Hestion- E500 Germany), Leuckhart molds, Digital rotary microtome (Hestion ERM 4000 Germany), Hot plate, Water Bath (Gallenkamp) Muslin cloth, Staining rack, Forceps and Swift.

3.4 COLLECTION OF EXPERIMENTAL PLANT MATERIALS

Experimental material i.e. *Parkia biglobosa* (Locust beans) was bought from Oregbeni market, Ikpoba hill, Benin City, Edo State.

3.4.1 IDENTIFICATION AND AUTHENTICATION

Prof. Aigbokhan Emmanuel Izaka of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, identified and authenticated the *P. biglobosa*. Following that, a sample plant was placed in the departmental herbarium, and a voucher number (UBH-P429) was given for referral.

3.4.2 PREPARATION OF PLANT MATERIAL

P. biglobosa was oven dried at 50⁰c using the Labtech oven for three days and pulverized to a fine powder with the use of a household blender. 3.016kg of fine powdered fermented *P. biglobosa* seed was obtained. Extraction was carried out using the soxhlex extractor, 14g of *P. biglobosa* was loaded into the thimble. Following this, 250ml of ethanol is added to the round bottom flask attached to the condenser and soxhlet extractor. The side arm is lagged with cotton wool. Using the isomantle to heat the ethanol, it evaporates and travels along the apparatus to the condenser. Following that, the condensate trickles into the reservoir holding the thimble. When the solvent volume reaches the siphon, it pours back into the flask, restarting the same procedure. This process should take 16 hours to complete. With the aid of a freeze-drying machine the extract was separated from the ethanol by evaporation. 77g of the fermented *P. biglobosa* seed extract was gotten.

3.5 DESCRIPTION AND PREPARATION OF DRUGS

Aspirin was gotten from a government-approved pharmaceutical store opposite the University of Benin Teaching Hospital, Ugbowo Road, Benin city; Edo State. Aspirin was diluted using 10ml of commercially purchased UNIBEN table water with NAFDAC number 014597 for 2 tablets containing 75mg of aspirin each.

3.6 ANIMAL HOUSING

Thirty male Wistar rats weighing between 136-240g was obtained from the animal housing facility of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City. Animals was housed in plastic cages with wire gauge and left to acclimatize for 14 days under standard conditions of temperature, and relative humidity $26^{\circ}\text{c} \pm 2^{\circ}\text{c}$ and 50% respectively. A 12:12hour natural light/dark cycle before the commencement of the experiment. The environment was kept clean and aerated and maintained regularly by the animal housekeeper. All animals was fed with standard pellets (standard feed Nigeria Plc.) and water ad libitum.

3.7 ETHICS

Experimental rats were handled according to international guidelines for handling experimental animals as reported by the Institute for Laboratory Animal Research (NRC, 1996).

3.7.1 ETHICAL APPROVAL

Approval for this research was sought from the Ethics and research committee Ministry of Agriculture, Edo State, Nigeria. The voucher number V.1041/89 was given.

3.8 METHODOLOGY

3.8.1 LETHAL DOSE DETERMINATION (LD50)

The lethal dose of fermented *P.biglobosa* seed extract was conducted to determine the appropriate dose to be administered to assay for effect of the extract on drug induced gastro toxicity. This was carried out using 6 albino Wistar rats divided into three groups of two rats per group. Different concentrations of fermented *p. biglobosa* seed extract, 3000mg/kg, 4000mg/kg and 5000mg/kg of the albino rats. They were observed for 48 hours for signs of adverse effect including death. At the highest dose of 5000mg/kg, no adverse effect was seen.

3.8.2 EXPERIMENTAL DESIGN

Animals were grouped into six groups having five rats per group (n=5). Group one serves as the control fed with pellet and water only, group two served as the positive control where fermented *P. biglobosa* seed extract was administered at 1000mg/kg. Group three served as the negative control, administered 20mg/kg of aspirin. Group four was administered 1000mg/kg of the extract and 20mg/kg of aspirin simultaneously. Group five was administered 1200mg/kg of extract for a week and 20mg/kg of aspirin administered the subsequent week. Group six was administered 20mg/kg of aspirin for a week and then 1500mg/kg of extract for the following week. All animals were given the prescribed dosage orally using a syringe and gauge measuring 5 ml.

After treatment for a total of 14 days, the animals were sacrificed using chloroform, a mild anesthetic. From the stomach to the chest wall, all animals were incised. Experimental rats' oesophagus, stomach, duodenum, and rectum were removed, cleaned in normal saline, and then fixed in 10% formalin to preserve the tissue architecture for histological research.

Table 3.1: EXPERIMENTAL PLAN

S/N	GROUPING	ADMINISTRATION
I	5	Control - 0mg/kg
II	5	Positive control - dose of 1000mg/kg of extract.
III	5	Negative control - dose of 20mg/kg of aspirin.
IV	5	Dose of 1000mg/kg of extract and 20mg/kg of aspirin administered simultaneously.
V	5	Dose of 1200mg/kg of extract administered the first week and 20mg/kg of aspirin administered the second week.
VI	5	Dose of 20mg/kg of aspirin administered the first week and 1500mg/kg of extract administered the second week.

3.8.3 GROSSING AND HISTOLOGY

The removed gastro-intestinal tissues were divided into slabs that are approximately 5 mm thick. They were handled by an automated tissue processor employing the paraffin wax procedure. The rotary microtome was used to cut sections to a thickness of 3 microns.

3.8.4 PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

- The sections were deparafinized in two changes of xylene for 2 minutes each.
- Tissue sections were taken through descending grades of alcohol. From absolute alcohol for 2 minutes, to 90% alcohol for 1 minutes, 70% alcohol for 1 minutes.
- The slides were rinsed in running tap water for one minute.
- Tissue sections were stained in hematoxylin for 10 minutes
- Tissue sections rinsed in distilled water for 30 seconds.
- Tissue sections were differentiated in 1% acid alcohol for 15 seconds
- The sections were rinsed in distilled water for 5 minutes.
- The sections were counter stained with 1% eosin for 5 minutes.
- The sections were washed in running tap water for 30 seconds.
- The sections were dehydrated by passing through ascending grades of alcohol (70%, 90%, 100%) for 1 minute each.
- The sections were cleared in two changes of xylene for 2 minutes each.
- The sections were mounted with DPX and viewed microscopically using objective lenses (Omorodion *et al*, 2017).

3.8.5 MICROSCOPY AND PHOTOMICROGRAPHY

At 100 and 400 magnifications, sections were examined using an Olympus binocular microscope with an integrated illumination system. After that, the sections were photomicrographed under a microscope with an Olympus trinocular microscope linked to a digital camera.

3.8.6 STATISTICAL ANALYSIS

The statistical analysis was conducted using Statistical programs for Social Sciences (SPSS) version 20.

CHAPTER FOUR

RESULTS

4.1 STATISTICAL ANALYSIS

Statistics showing the mean weight of the stomach, initial and final weight and the graph of stomach weight.

Table 4.1: Weight of stomach, initial and final body weight of all groups after administration

Parameters	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
Stomach weight	104.7 ± 1.86	105.0 ± 1.53	104.0 ± 2.08	103.7 ± 0.88	103.0 ± 0.58	105.7 ± 0.88
Initial weight	0.767 ± 0.03	0.667 ± 0.03	0.7333 ± 0.03	0.767 ± 0.033	0.733 ± 0.07	0.767 ± 0.03
Final weight	1.800 ± 0.17	1.367 ± 0.067	1.500 ± 0.12	1.633 ± 0.03	1.467 ± 0.27	1.700 ± 0.10

$P < 0.05$ indicates significant difference

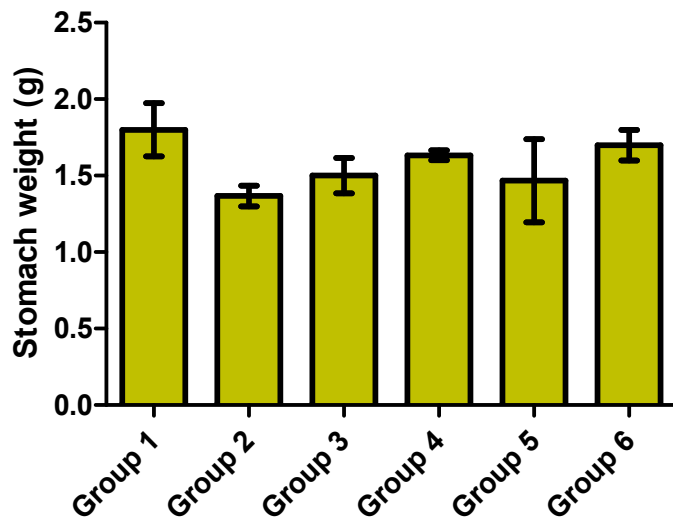


Figure 4.1: Graph of stomach rate after sacrifice

There were no significant differences in group 2, group 3, group 4, group 5 and group 6 compared with group 1 respectively.

4.2 HISTOLOGICAL ANALYSIS

Photomicrography of the oesophagus, stomach, small intestine and large intestine of group 1 to group 6

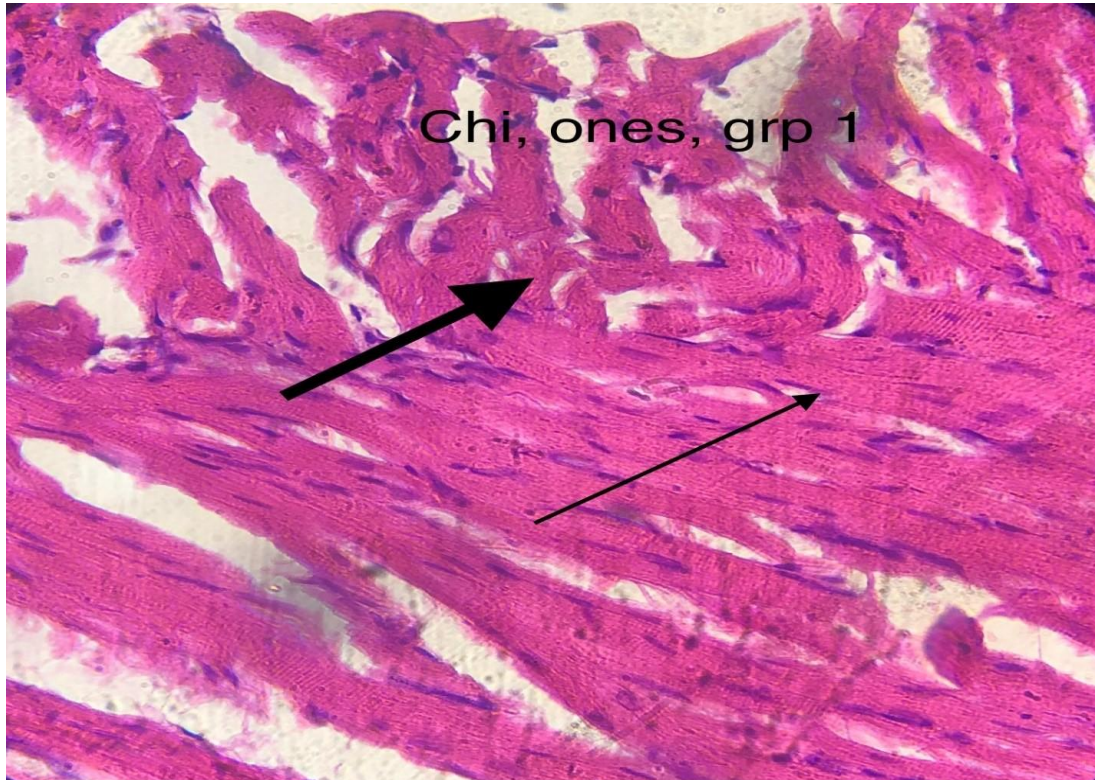


Plate 4.1: Photomicrograph of tissue esophagus of with normal mucosa, submucosa and muscularis (arrows) rat which was administered only rat pellet and distilled water and serve as control. H & E. X400mag.

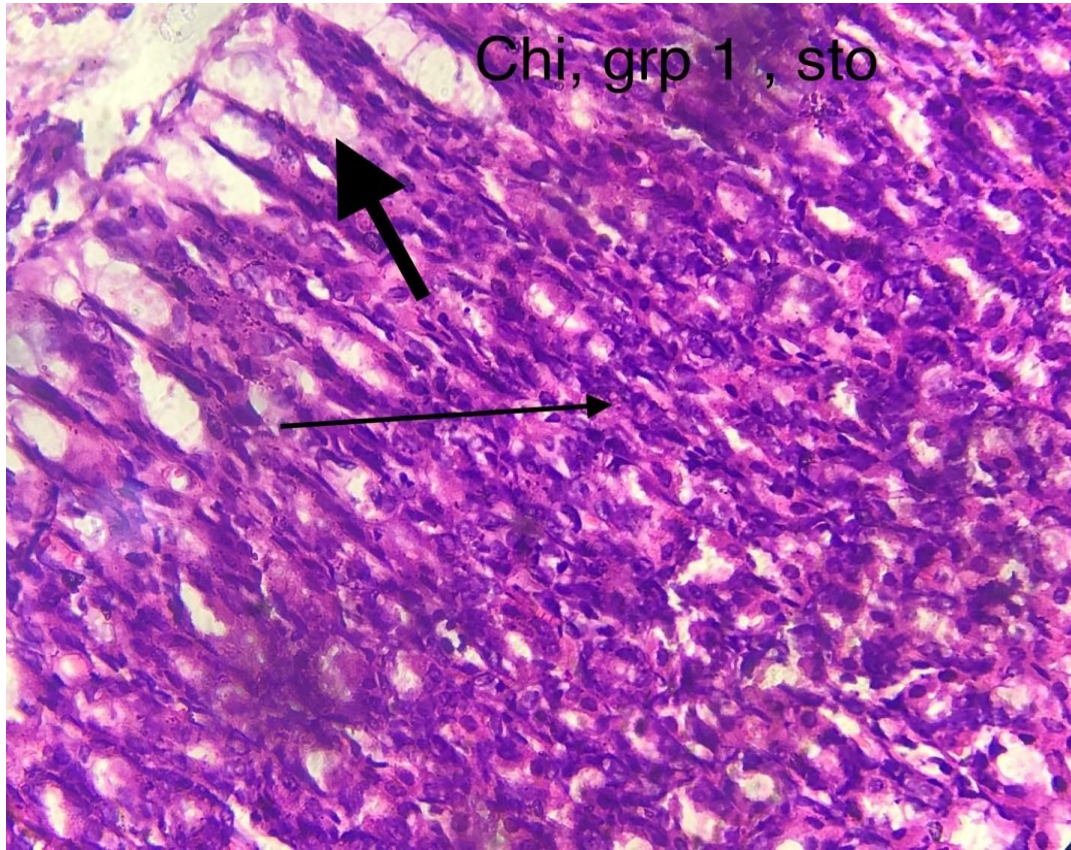


Plate 4.2: Photomicrograph of tissue distal part of the stomach with normal gastric pits, goblets cells, mucosa and submucosa area (arrows) rat which was administered only rat pellet and distilled water and serve as control. H & E. X400mag.

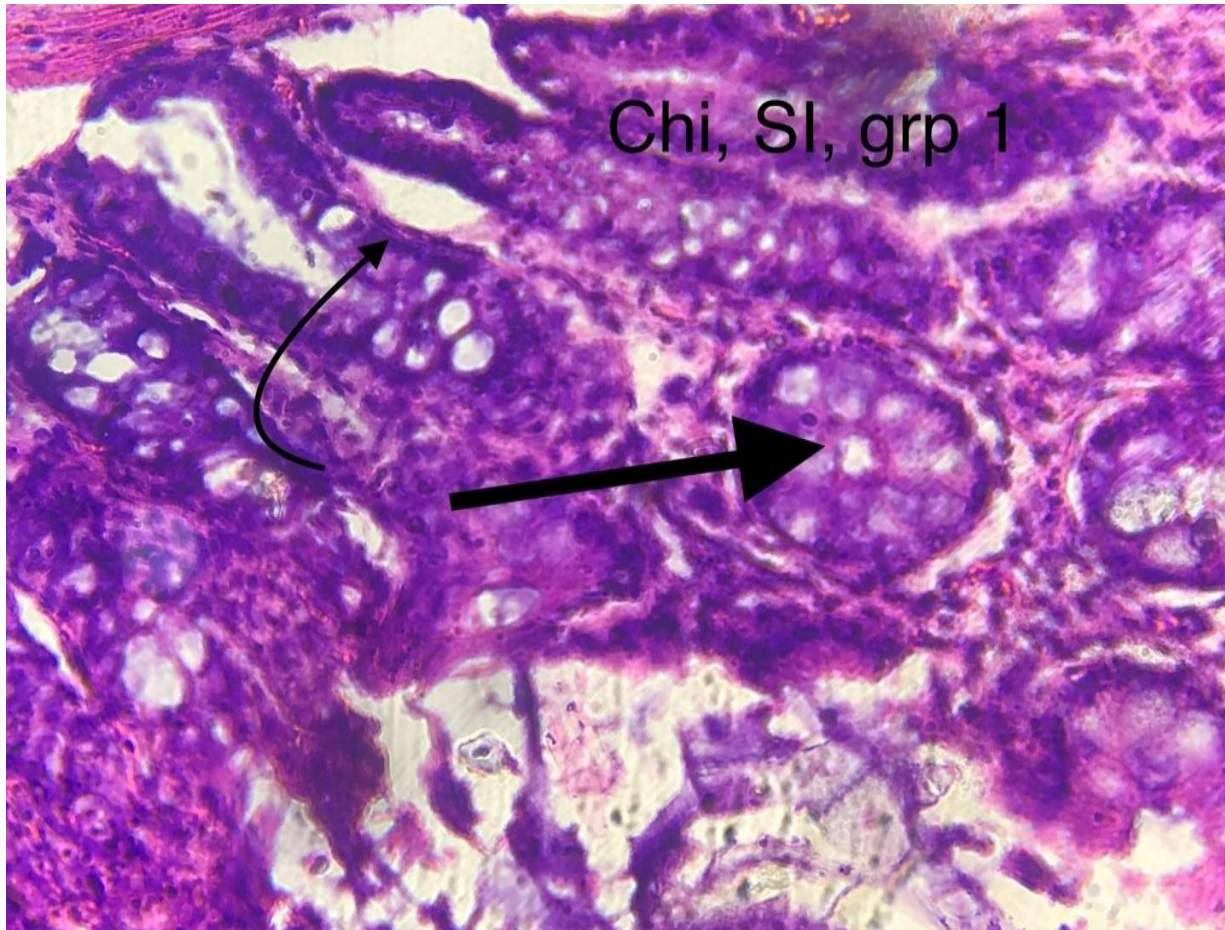


Plate 4.3: Photomicrograph of tissue distal part of the small intestinal tissue with normal villi, brunner's glands and goblets cells (arrows) rat which was administered only rat pellet and distilled water and serve as control. H & E. X400mag.

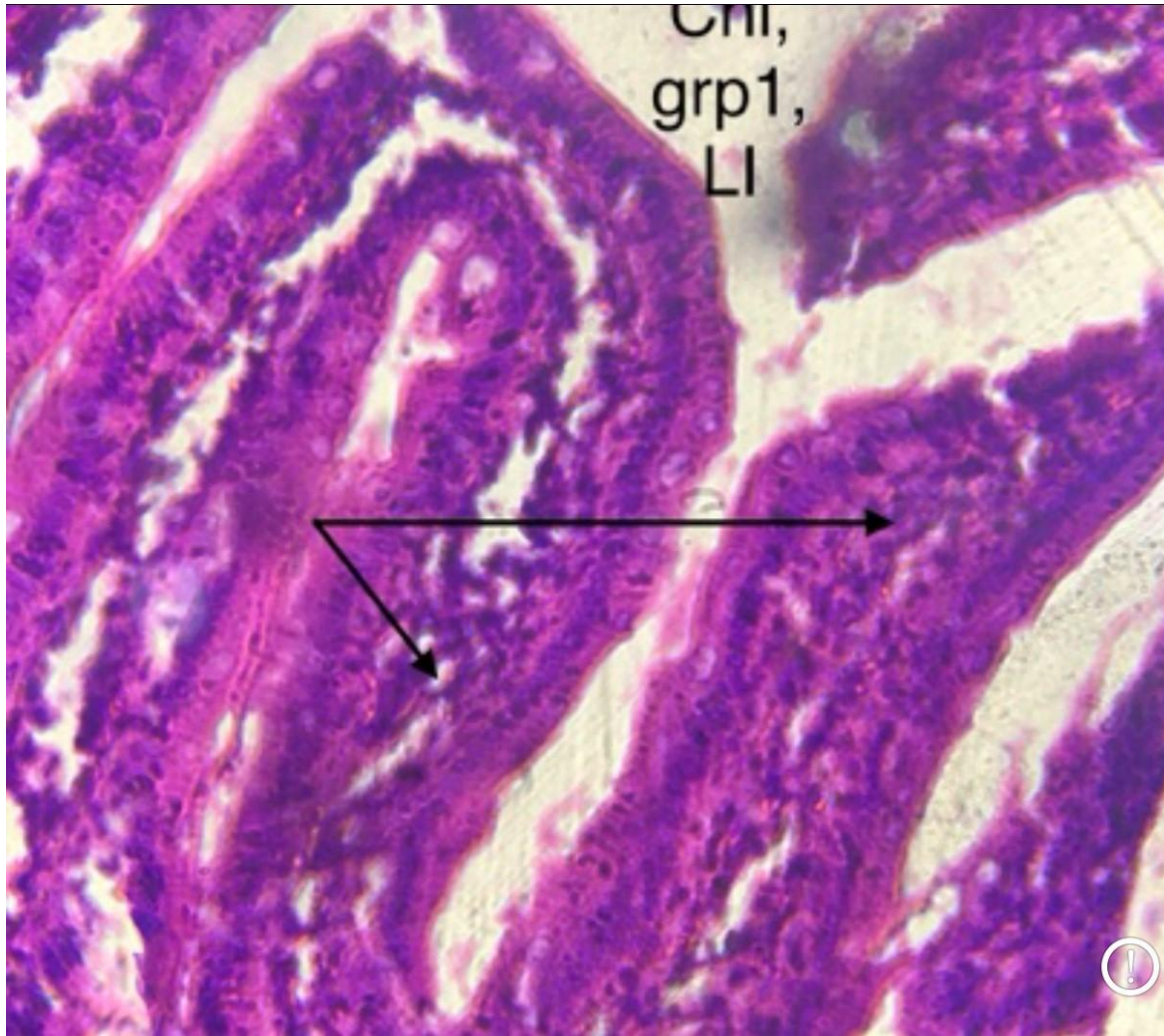


Plate 4.4: Photomicrograph of tissue the large intestinal tissue with normal crypts of lieberkuhn and panel cells (arrows) rat which was administered only rat pellet and distilled water and serve as control. H & E. X400mag.

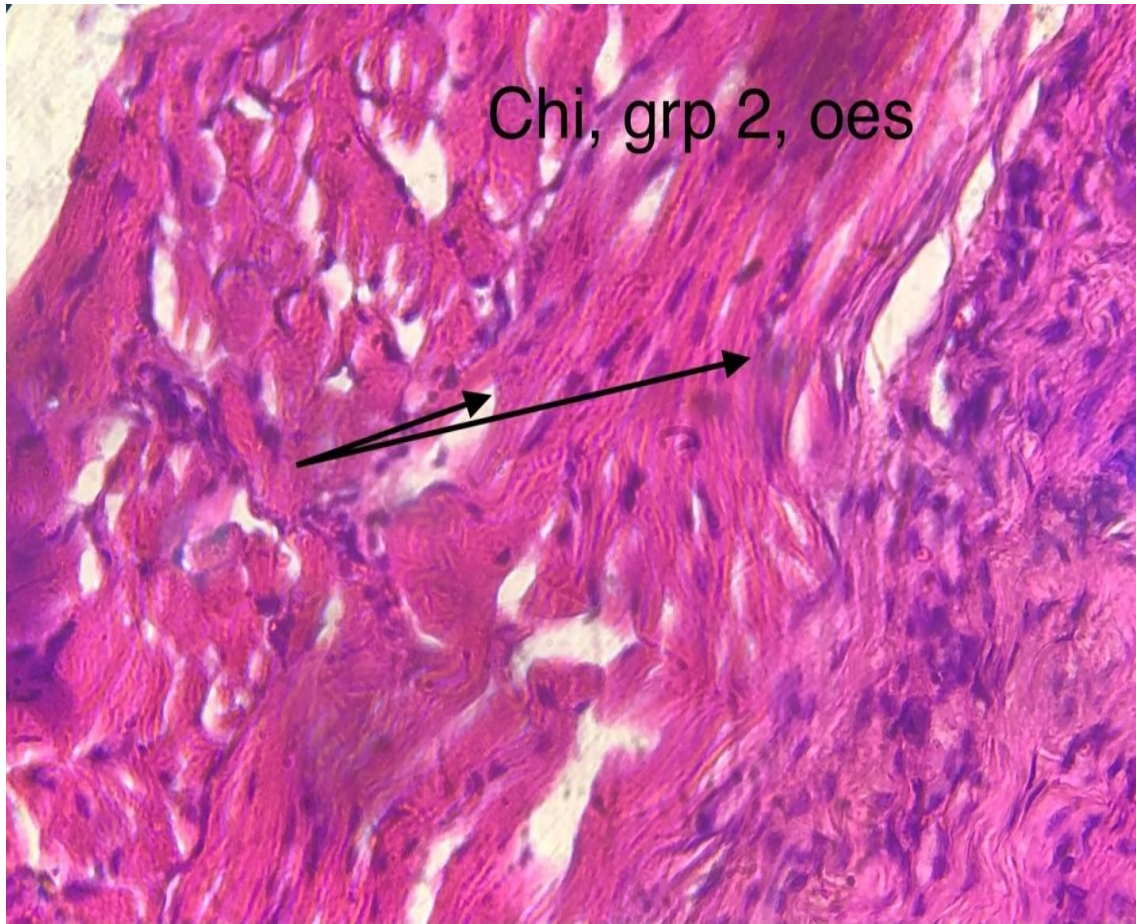


Plate 4.5: Photomicrograph of esophageal tissue with normal stratified squamous epithelia, mucosa and submucosa which received dose of 1000mg/kg of extract and served as positive control. H and E. X 400mag.

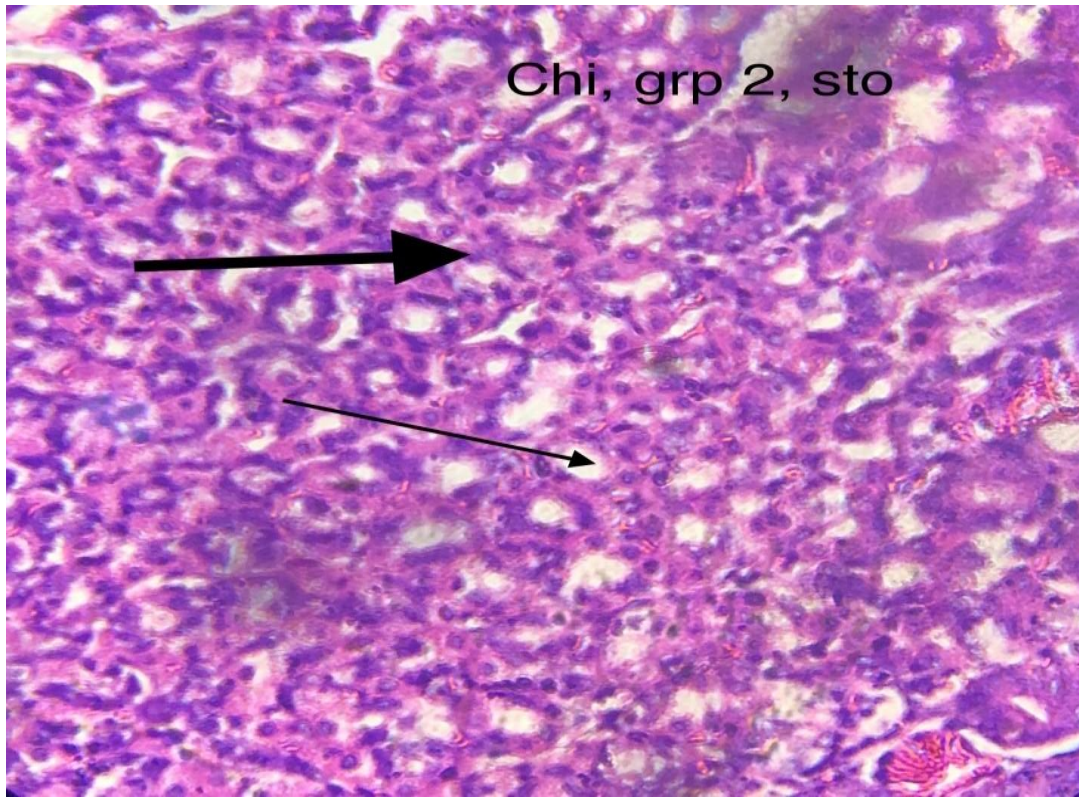


Plate 4.6: Photomicrograph of stomach tissue with normal gastric pits, goblet cells, gastric gland (thick and thin arrows) which received dose of 1000mg/kg of extract and served as positive control. H and E. X 400mag.

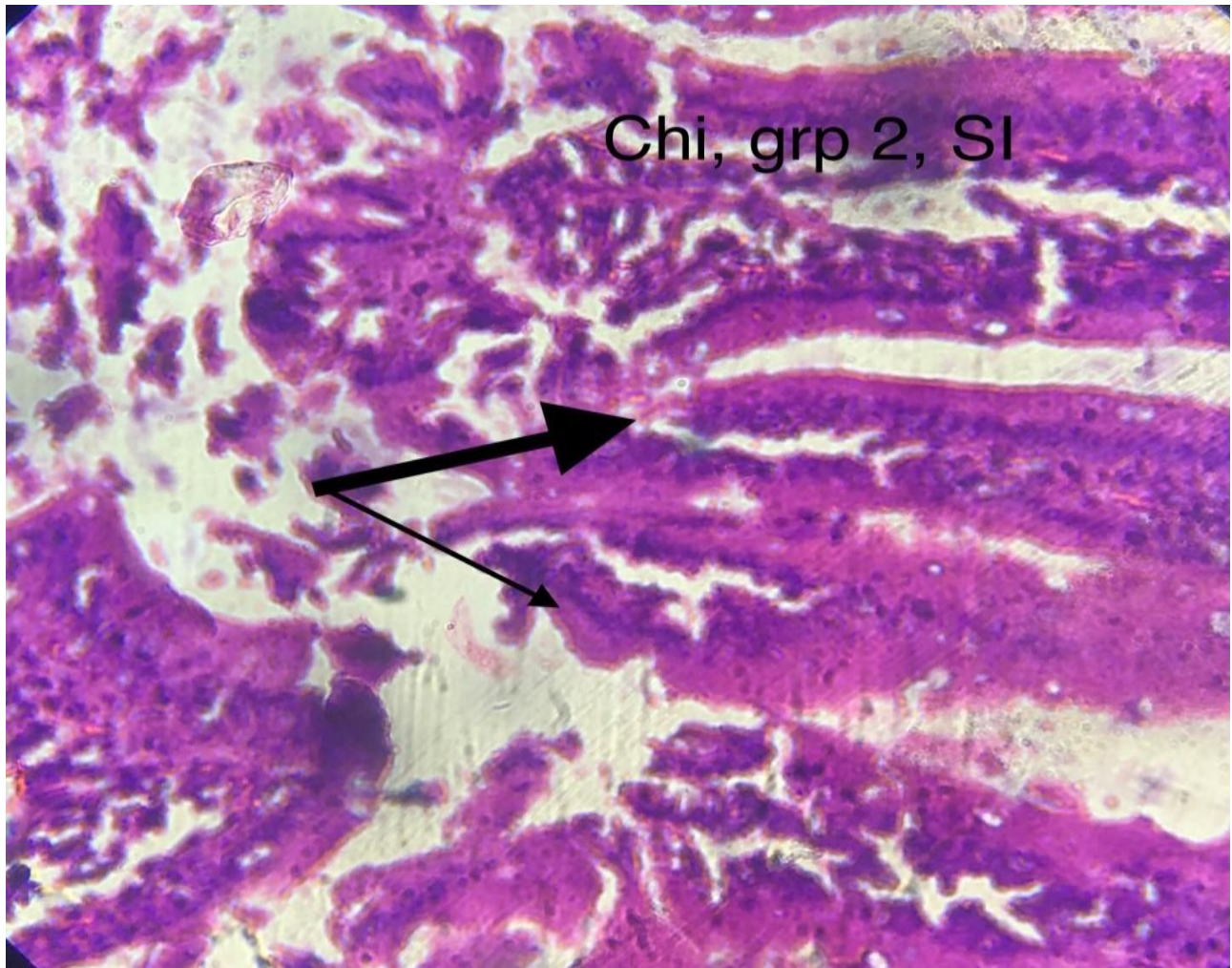


Plate 4.7: Photomicrograph of distal part of the small intestinal tissue with normal villi, panel cells and crypts of lieberkuhn (thick and thin arrows) which received dose of 1000mg/kg of extract and served as positive control. H and E. X 400mag

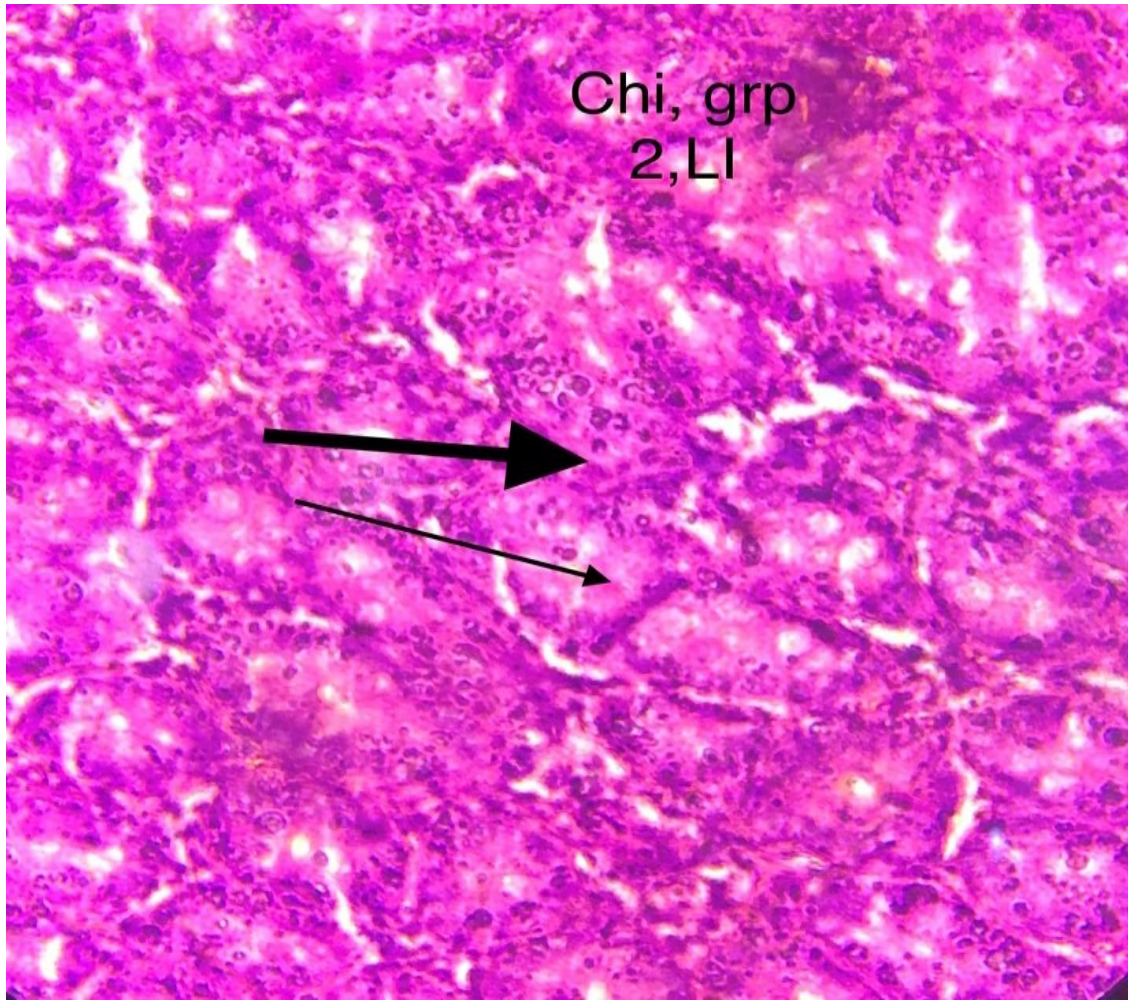


Plate 4.8: Photomicrograph of distal part of the large intestinal tissue with normal goblet cells, panel cells and crypts of lieberkuhn (thick and thin arrows) which received dose of 1000mg/kg of extract and served as positive control. H and E. X 400mag

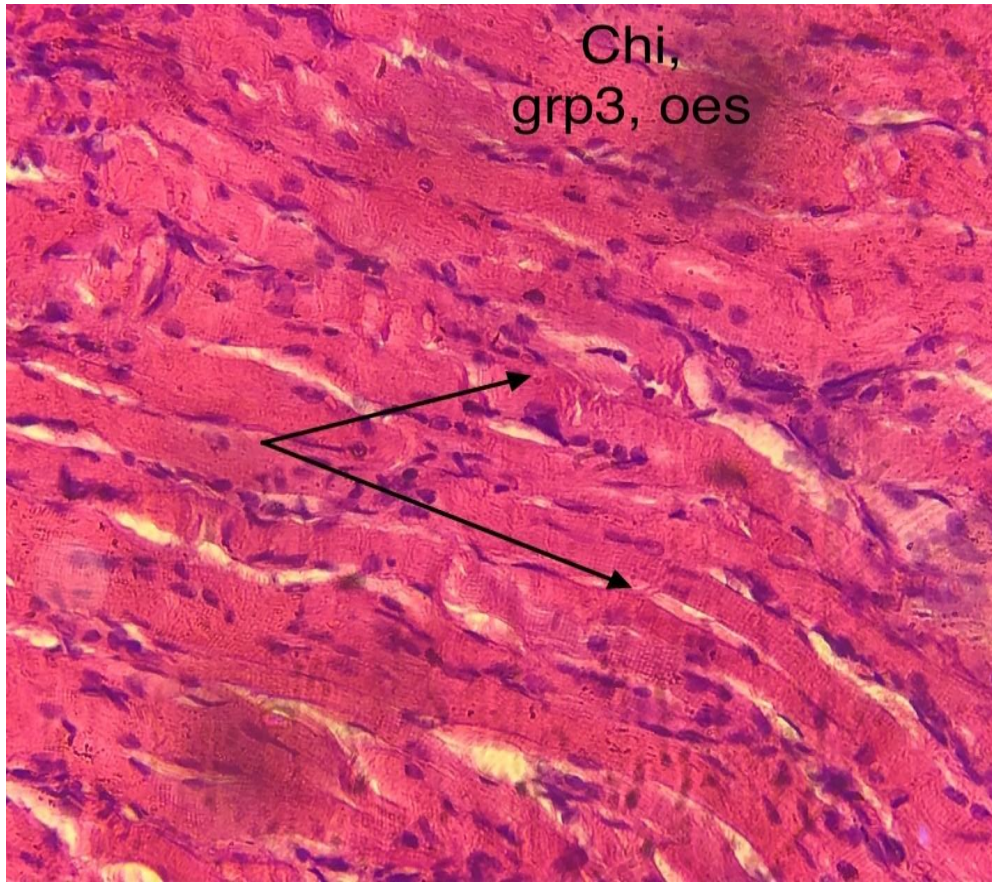


Plate 4.9: Photomicrograph of distal part of the esophageal tissue with slightly inflamed mucosa, submucosa and muscularis (thin arrows) which received dose of 20mg/kg of aspirin and served as negative control. H and E. X 400mag

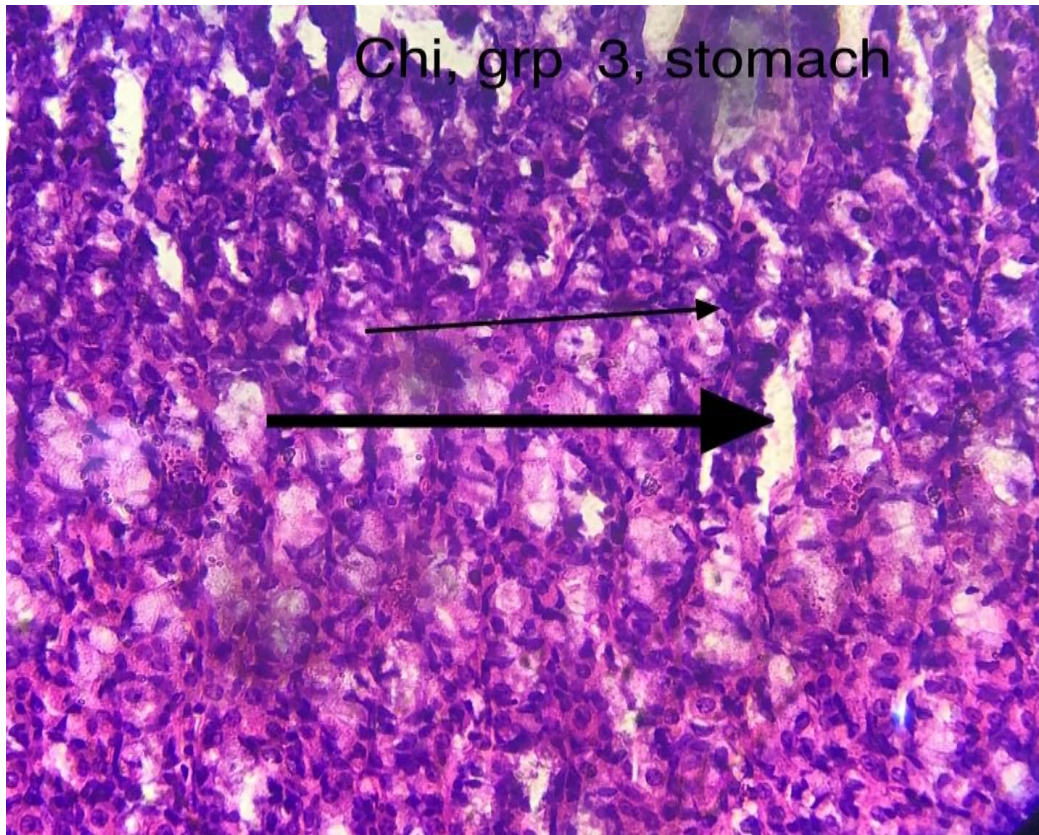


Plate 4.10: Photomicrograph of distal part of the stomach tissue with inflamed gastric glands and pits (gastritis)(thin arrows) which received dose of 20mg/kg of aspirin and served as negative control. H and E. X 400mag

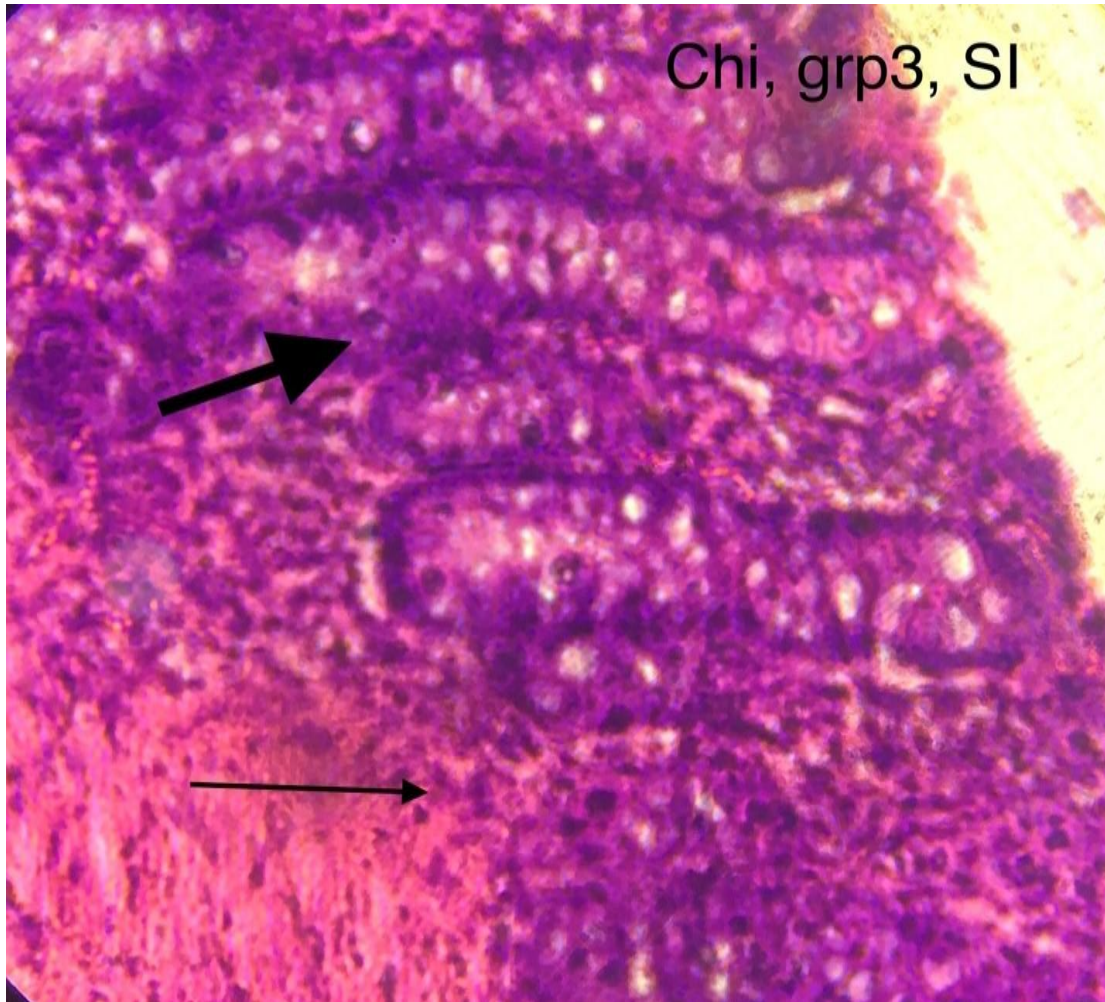
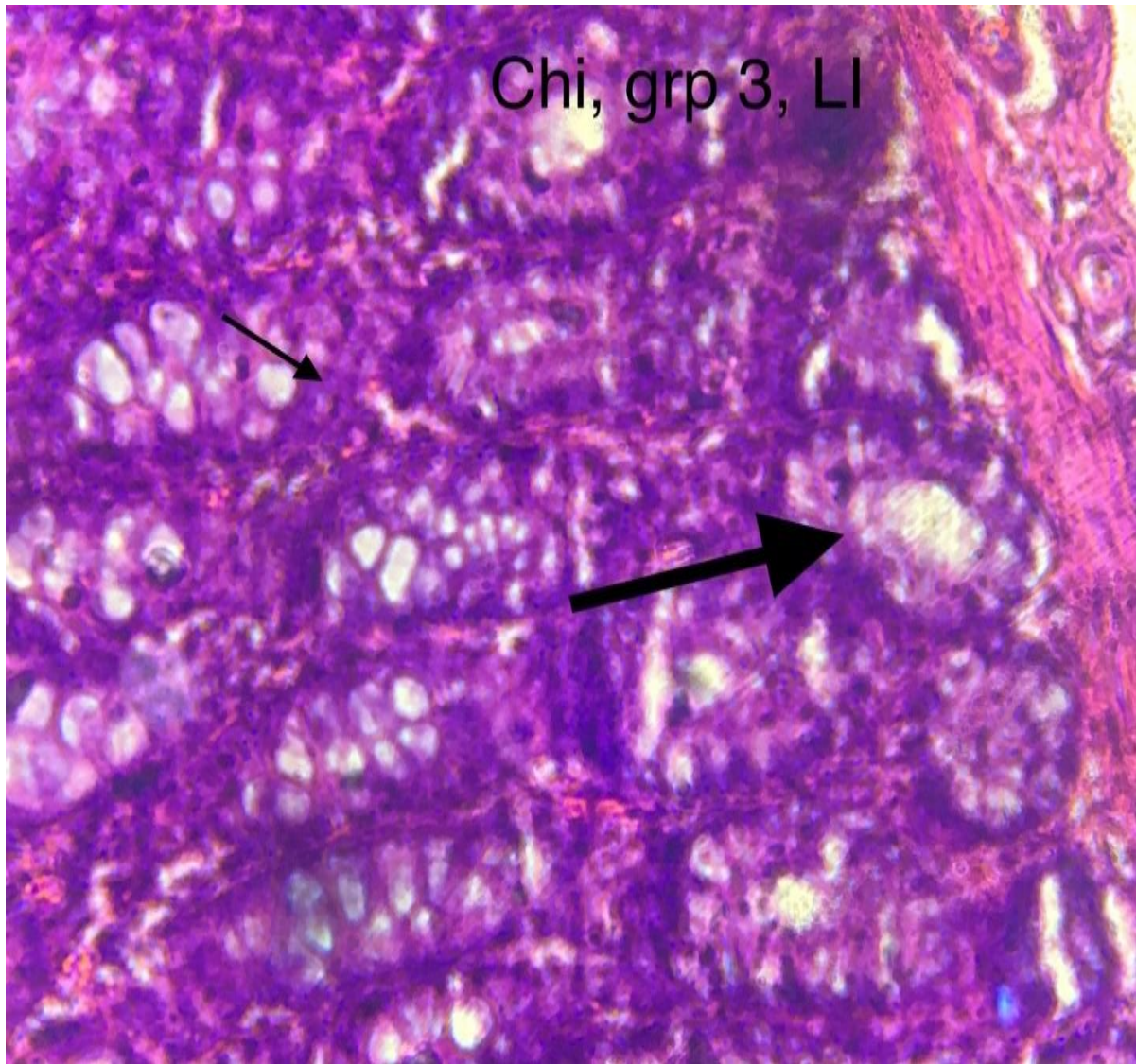


Plate 4.11: Photomicrograph of the small intestinal tissue with inflamed submucosa, paneth cells (thick and thin arrows) which received dose of 20mg/kg of aspirin and served as negative control. H and E. X 400mag



Plat

e 4.12: Photomicrograph of the large intestinal tissue with inflamed crypts of Lieberkuhn, goblet cells and Paneth cells (thick and thin arrows) which received a dose of 20mg/kg of aspirin and served as a negative control. H and E. X 400mag

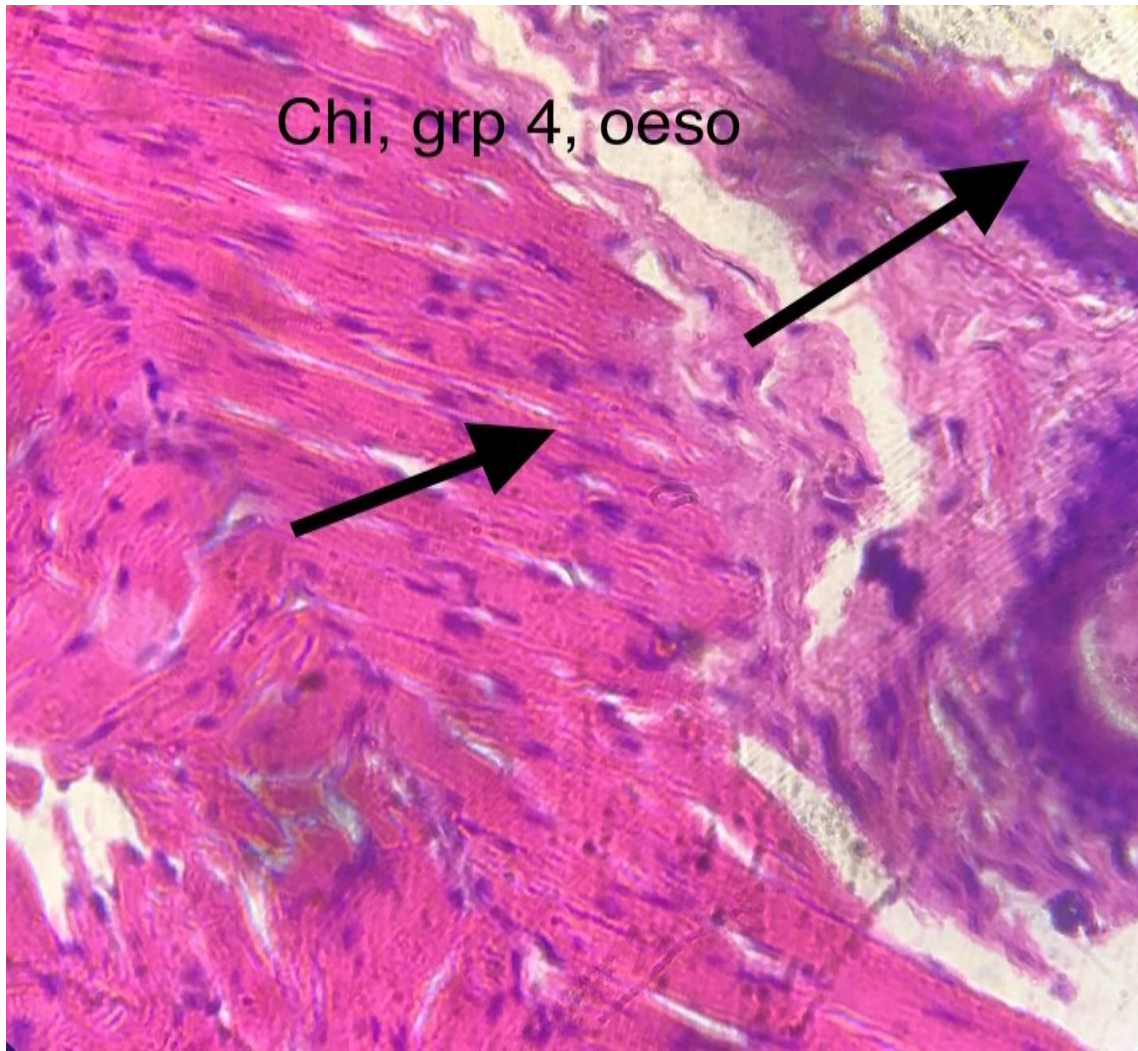


Plate 4.13: Photomicrograph of the esophageal tissue with normal stratified squamous epithelium, submucosa and muscularis (thick arrows) which received Dose of 1000mg/kg of extract and 20mg/kg of aspirin administered simultaneously (grp IV). H and E. X 400mag

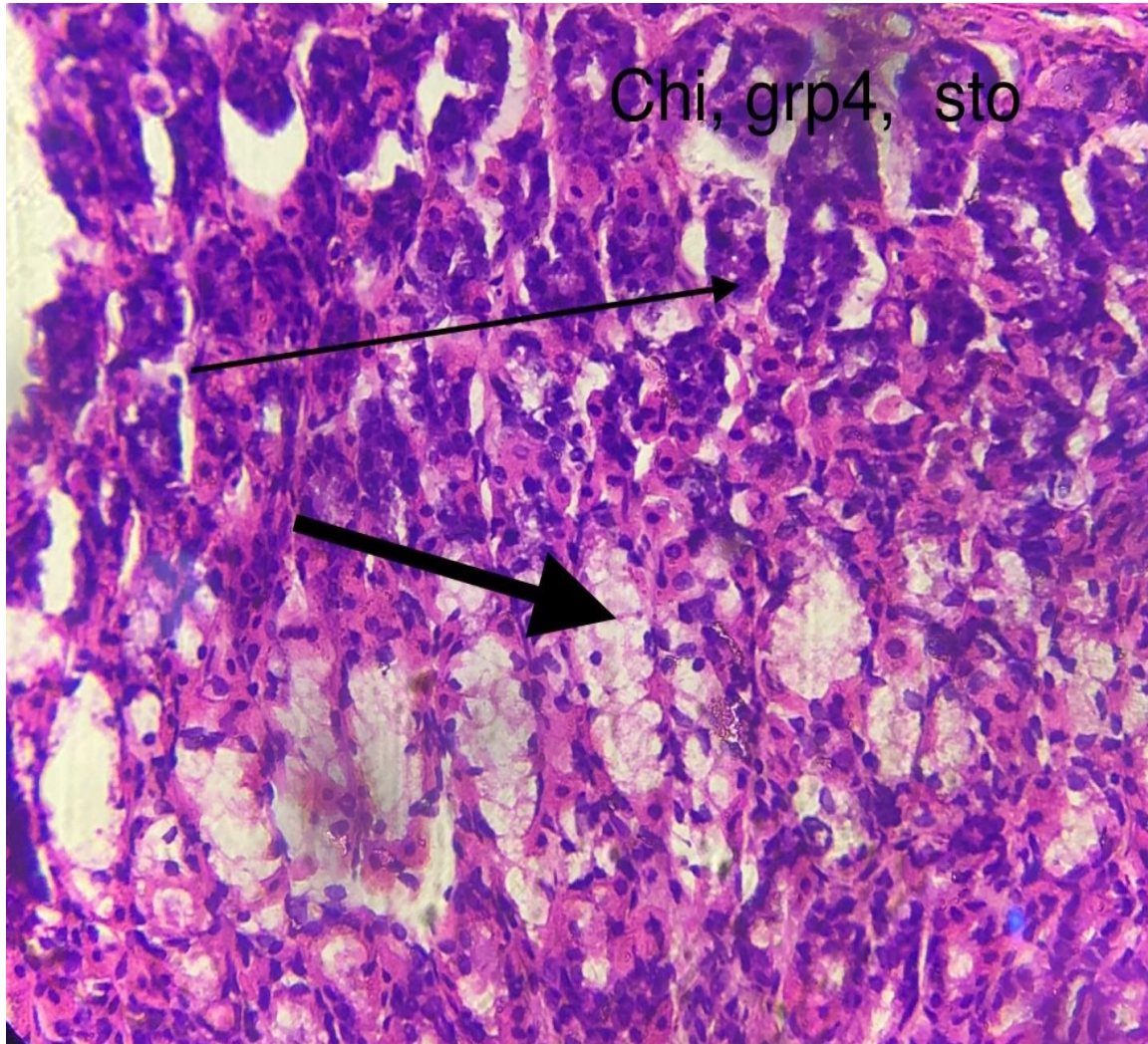


Plate 4.14: Photomicrograph of the stomach tissue with normal gastric pits and goblet cells (thick and thin arrows) which received Dose of 1000mg/kg of extract and 20mg/kg of aspirin administered simultaneously (grp IV). H and E. X 400mag

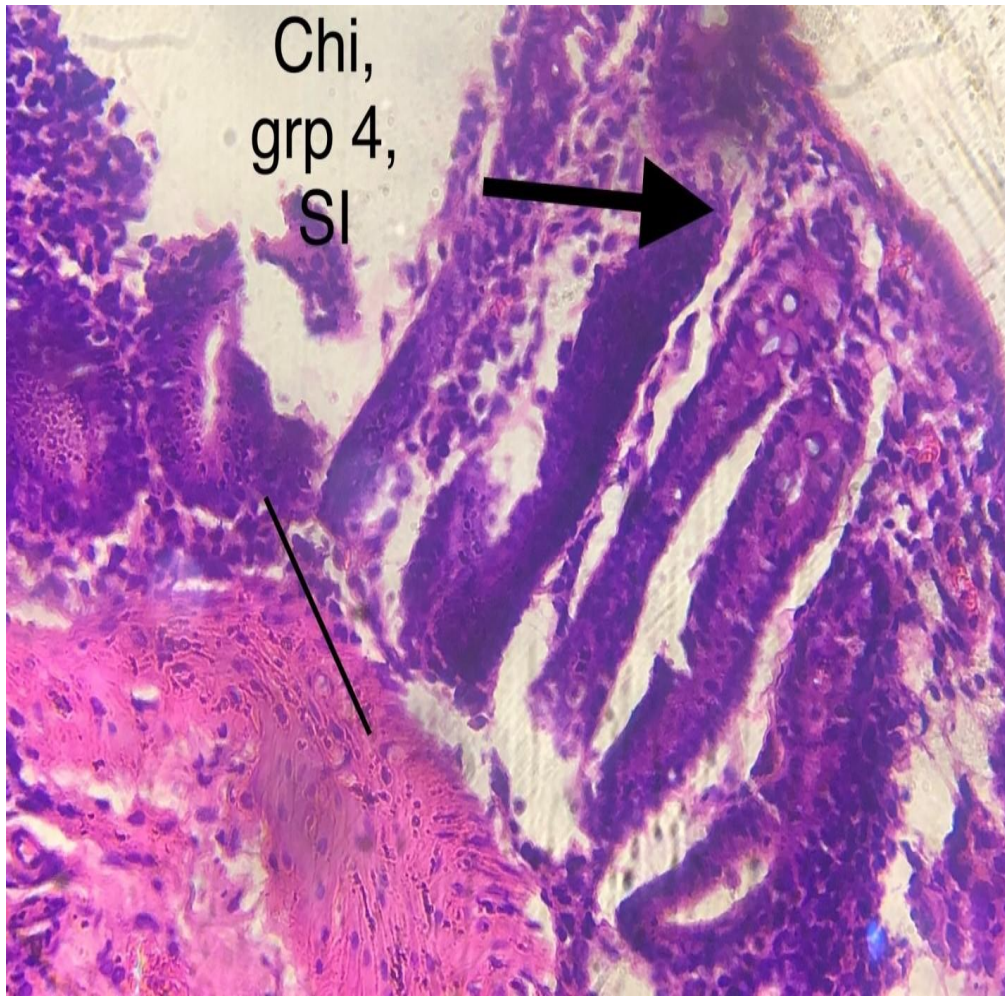


Plate 4.15: Photomicrograph of the small intestinal tissue with normal villi, paneth cells and goblet cells (thick and thin arrows) which received Dose of 1000mg/kg of extract and 20mg/kg of aspirin administered simultaneously (grp IV). H and E. X 400mag

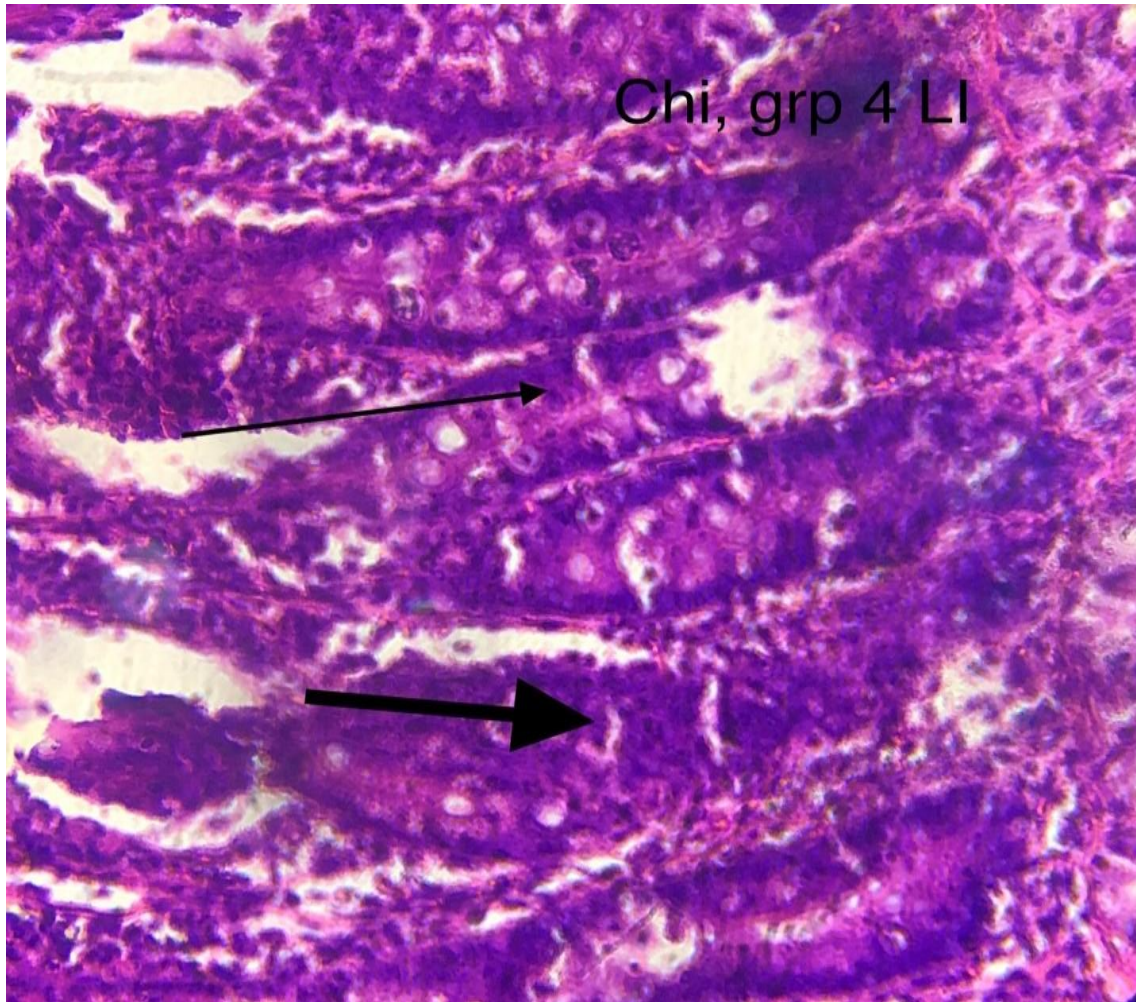


Plate 4.16: Photomicrograph of the large intestinal tissue with normal crypts of Lieberkuhn and goblet cells (thick and thin arrows) which received a dose of 1000mg/kg of extract and 20mg/kg of aspirin administered simultaneously (grp IV). H and E. X 400mag



Plate 4.17: Photomicrograph of the esophageal tissue with normal mucosa and submucosa (thick arrows) which received Dose of 1200mg/kg of extract administered the first week and 20mg/kg of aspirin administered the second week (group 5).H and E. X 400mag

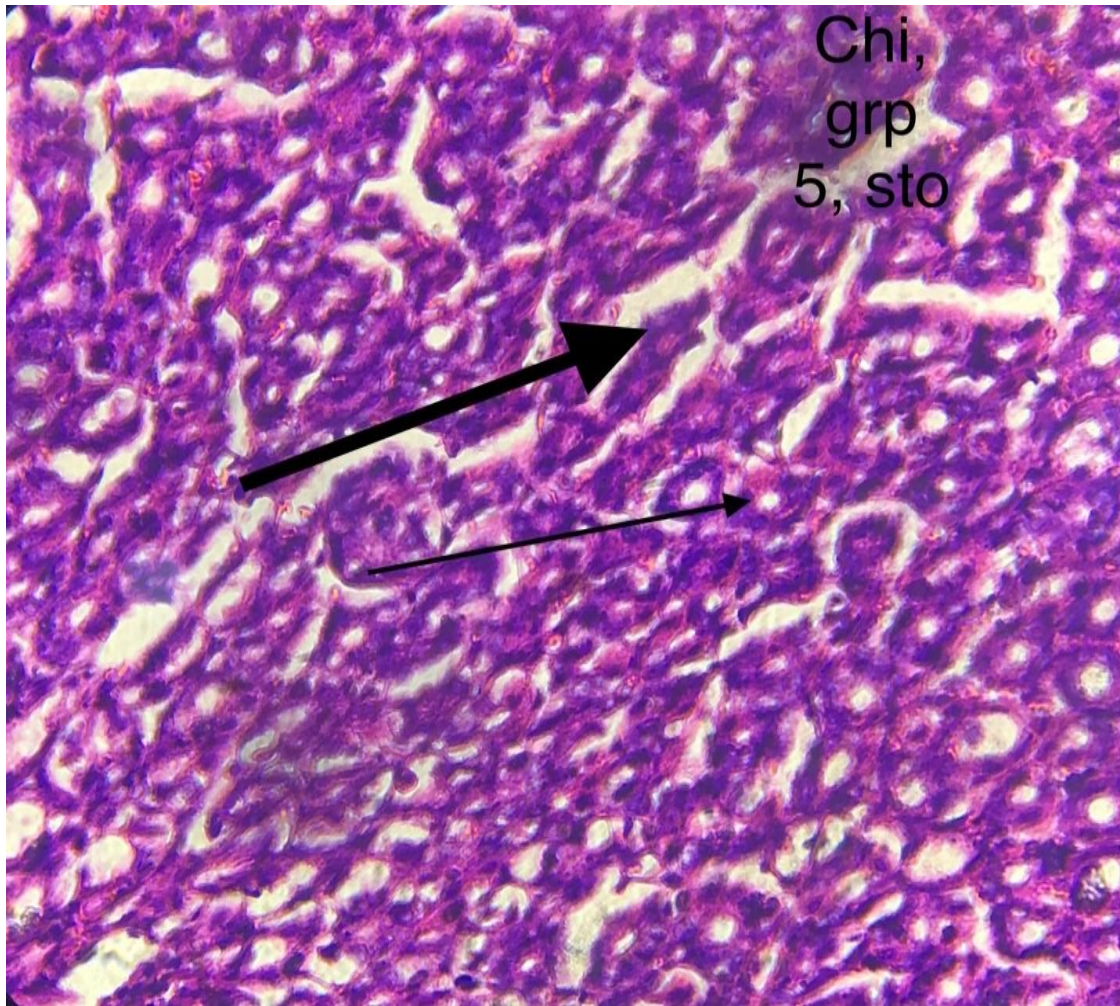
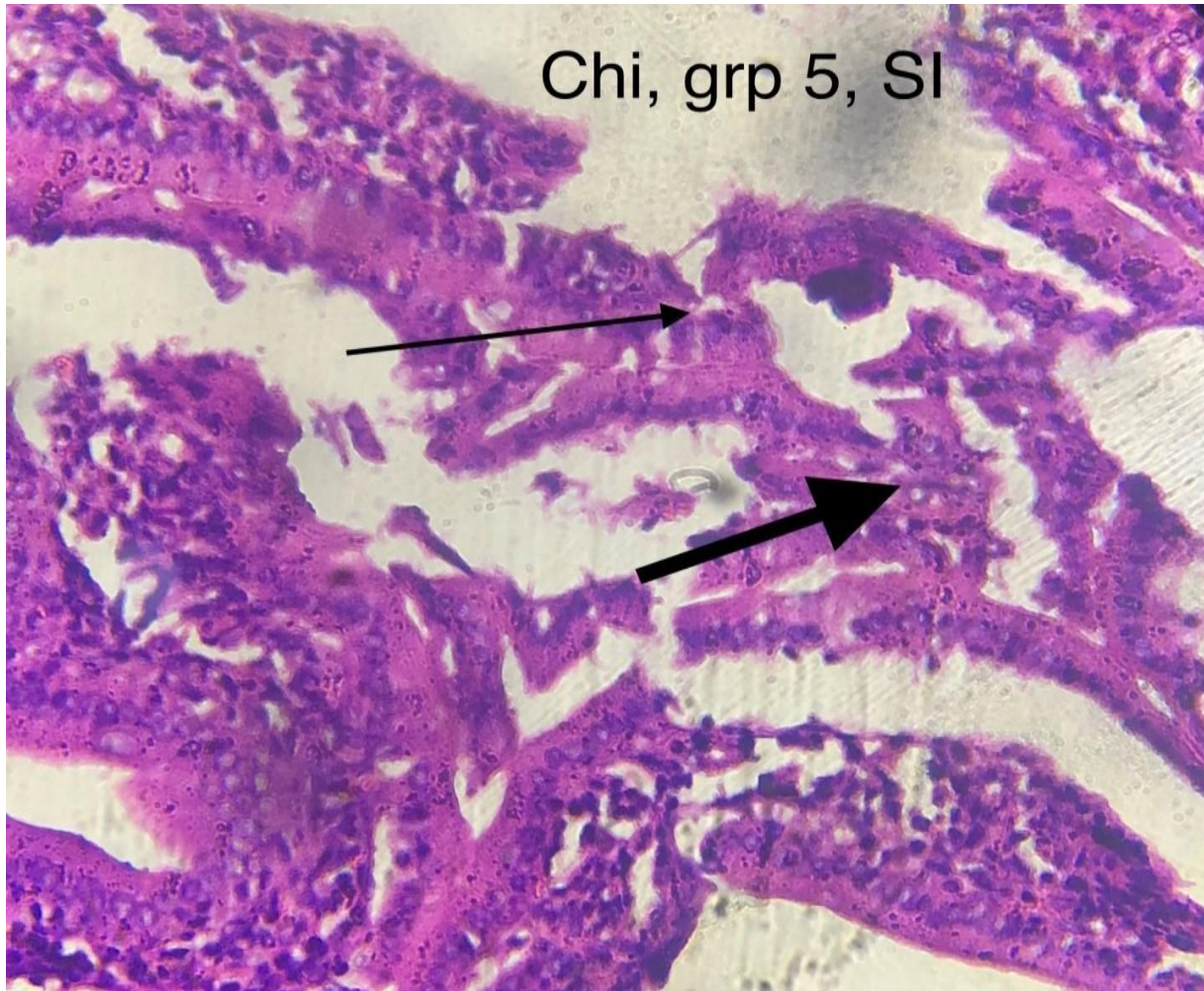


Plate 4.18: Photomicrograph of the Stomach tissue with slightly inflamed gastric pits and gastric glands (thin and thick arrows) which received Dose of 1200mg/kg of extract administered the first week and 20mg/kg of aspirin administered the second week (group 5).H and E. X 400mag



PI

ate 4.19: Photomicrograph of the small intestinal tissue with normal crypts of Lieberkühn, mucosa and Paneth cells (thin and thick arrows) which received a dose of 1200mg/kg of extract administered the first week and 20mg/kg of aspirin administered the second week (group 5). H and E. X 400mag

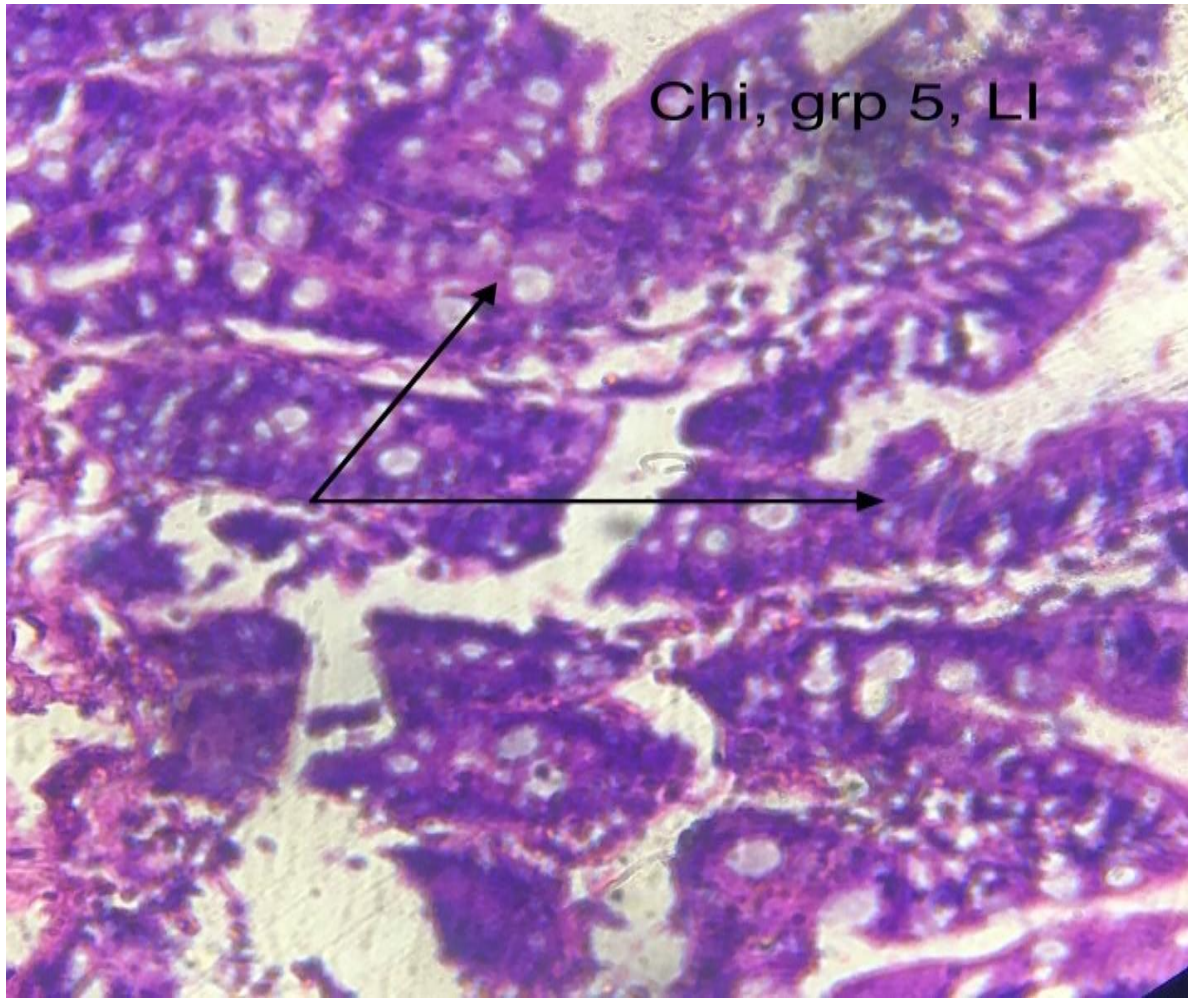


Plate 4.20: Photomicrograph of the large intestinal tissue with slightly inflamed crypts of lieberkuhn and goblets cells (thin and thick arrows) which received Dose of 1200mg/kg of extract administered the first week and 20mg/kg of aspirin administered the second week (group 5).H and E. X 400mag

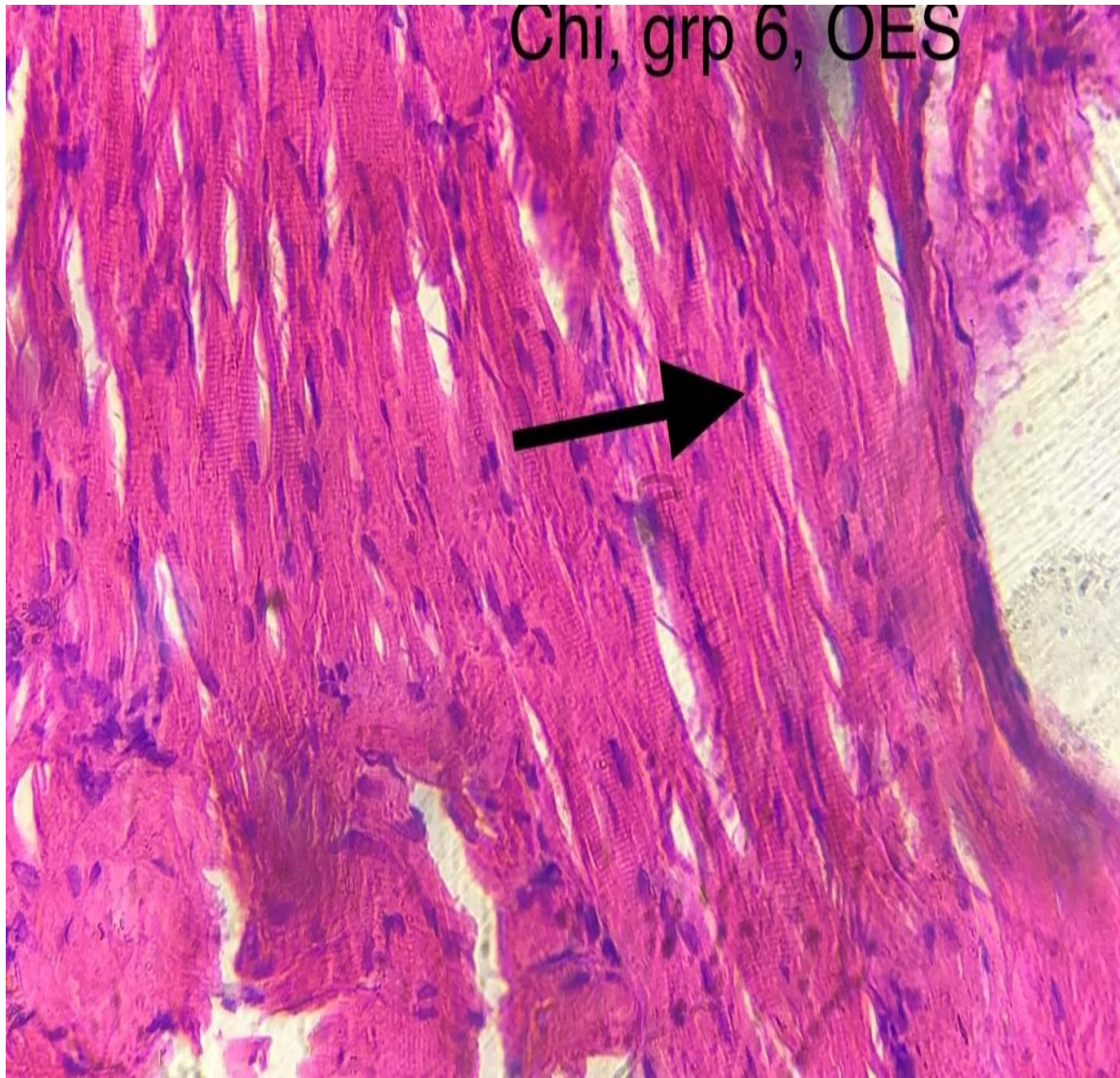


Plate 4.21: Photomicrograph of the esophageal tissue with normal mucosa, submucosa muscularis, and goblet cells (thick arrows) which received Dose of 20mg/kg of aspirin administered the first week and 1500mg/kg of extract administered the second week (group 6). H and E. X 400mag

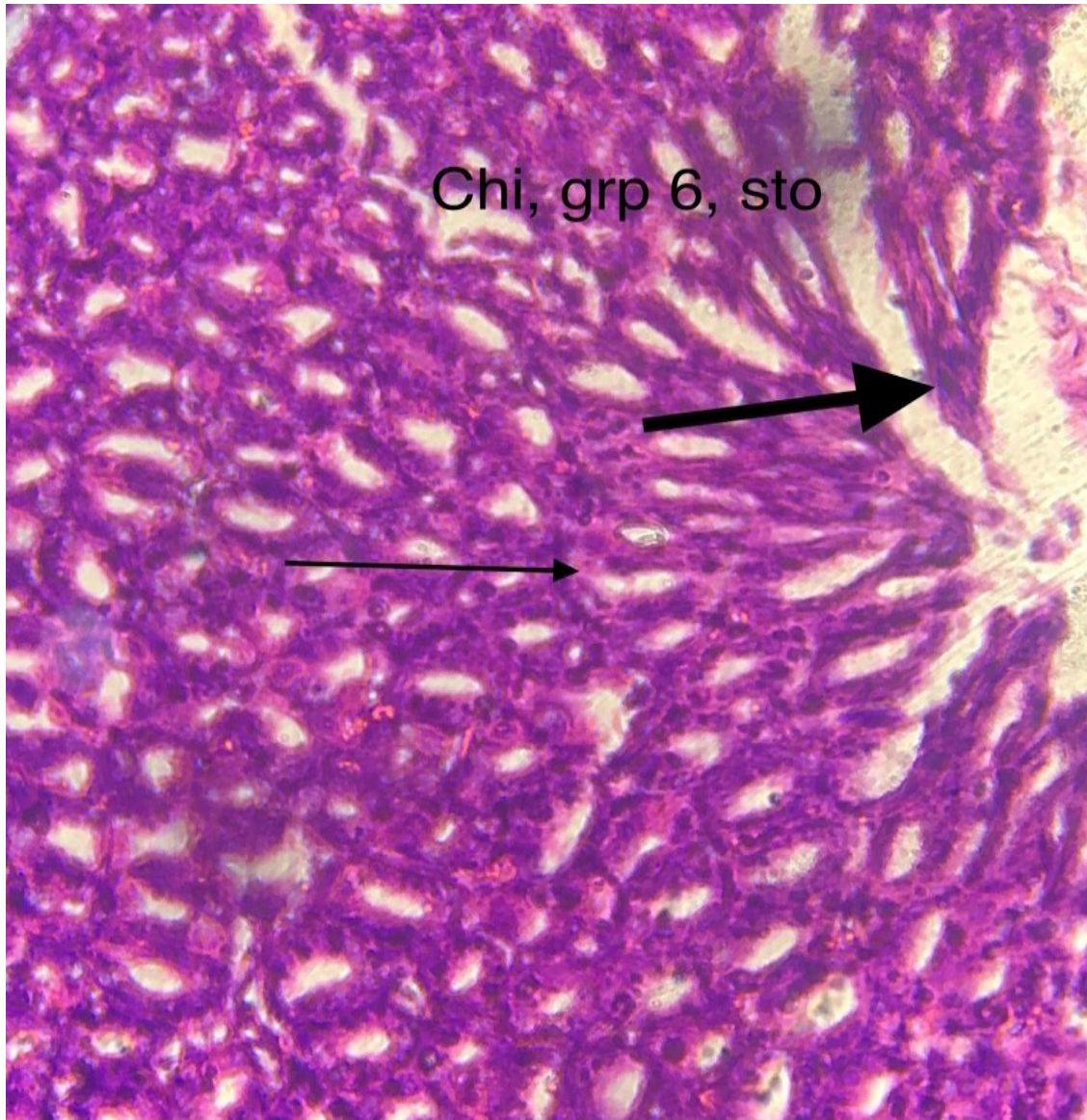


Plate 4.22: Photomicrograph of the stomach tissue with slightly inflamed gastric pits and goblet cells (thin and thick arrows) which received Dose of 20mg/kg of aspirin administered the first week and 1500mg/kg of extract administered the second week (group 6). H and E. X 400mag

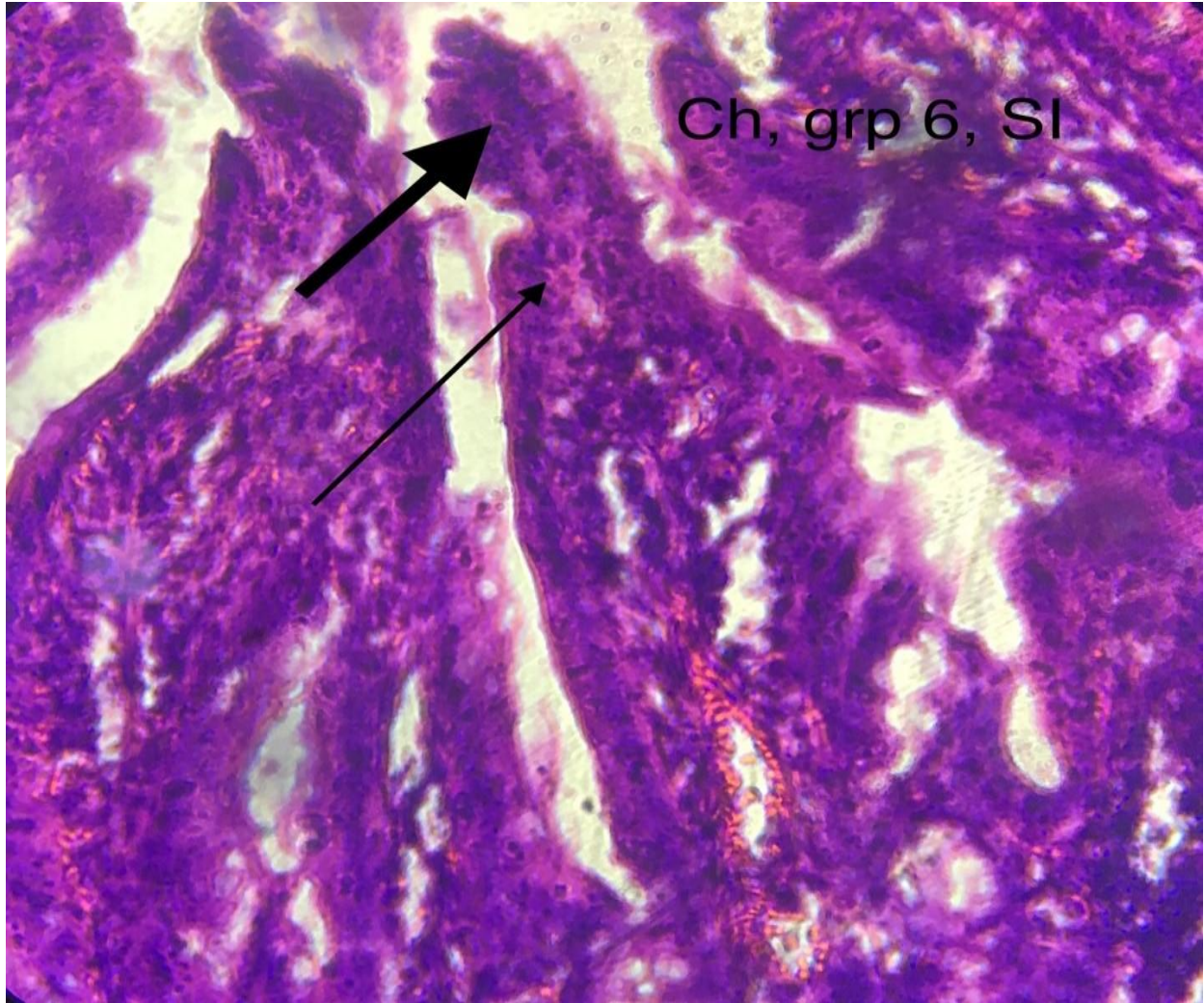


Plate 4.23: Photomicrograph of the small intestinal tissue with slightly inflamed crypt of Lieberkuhn and Paneth cells (thin and thick arrows) which received a dose of 20mg/kg of aspirin administered the first week and 1500mg/kg of extract administered the second week (group 6). H and E. X 400mag

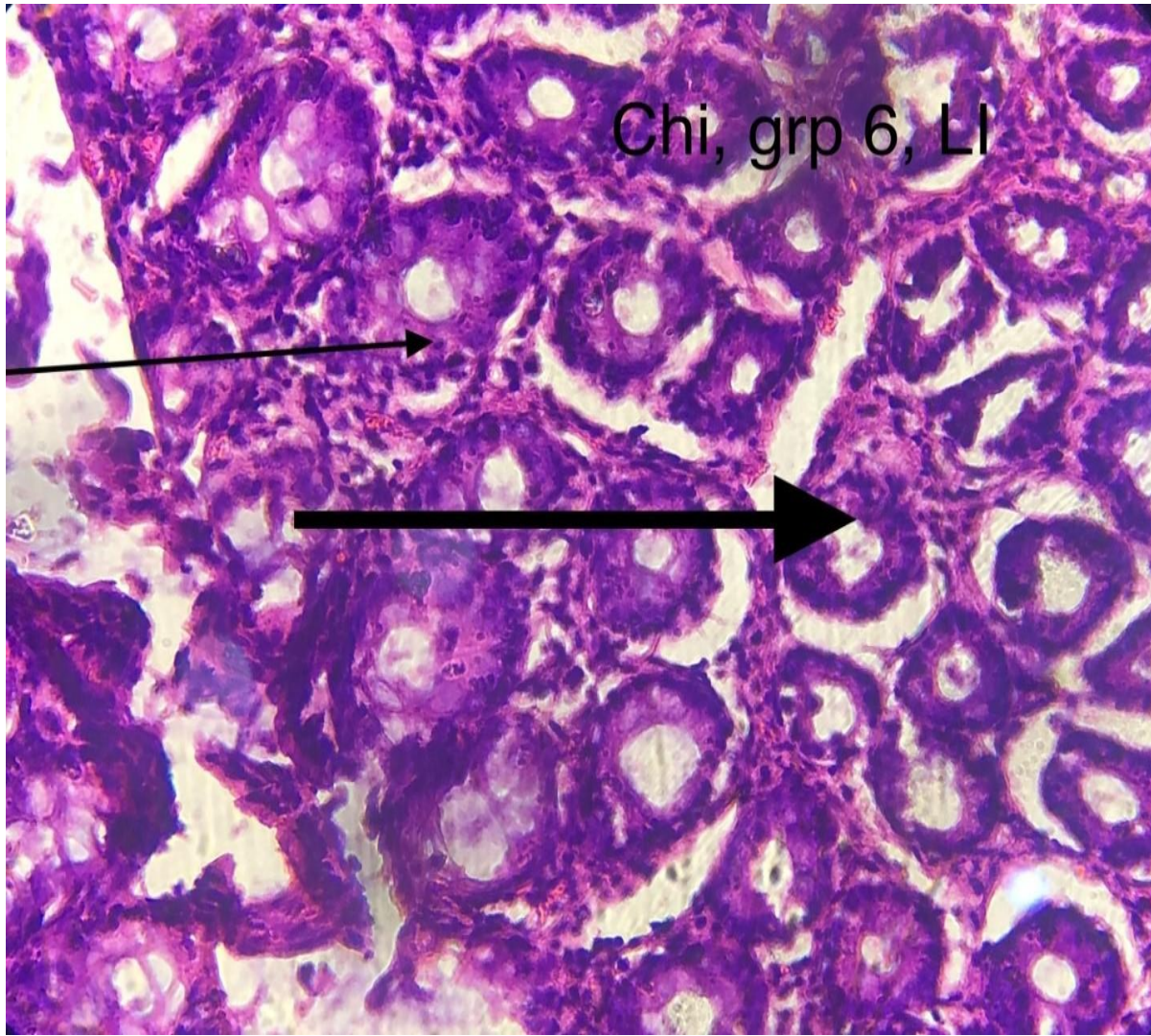


Plate 4.24: Photomicrograph of the large intestinal tissue with slightly inflamed mucose with crypts of lieberkuhn (thin and thick arrows) which received Dose of 20mg/kg of aspirin administered the first week and 1500mg/kg of extract administered the second week (group 6). H and E. X 400mag

CHAPTER FIVE

5.1 DISCUSSION

In the statistical analysis of the stomach weight, across all groups the mean weight value which were, 104.7 ± 1.86 , 105.0 ± 1.53 , 104.0 ± 2.08 , 103.7 ± 0.88 , 103.0 ± 0.58 and 105.7 ± 0.88 respectively were not significant with a p value <0.05 . The initial and final body weight mean for group one, two, three, five and six were not significant. Group four had a significant mean value of 0.767 ± 0.033 and 1.633 ± 0.03 for initial and final weight respectively with a p value of <0.05 . This is contrary to the study by Kebe *et al*, (2020), which studied "the protective effect of aqueous seed extract of *P.biglobosa* on the anterior pituitary gland of wistar rat", when compared to the initial body weight, the study's morphological observations demonstrate a discernible, substantial rise in the final mean body weight for the control, low dose and high dose group.

Group 1 which was fed only pellet and water and group two administered 1000mg/kg of *P.biglobosa* had normal histological features had no adverse effect. This was in accordance with the research carried out by Kebe *et al*, (2020), on "the protective effect of aqueous seed extract of *Parkia biglobosa* on the anterior pituitary gland of wistar rat", it was reported that fermented seed of *Parkia biglobosa* had no adverse effect on the anterior pituitary gland.

In the experiment, group 3 which was administered 20mg/kg of aspirin had inflammation in the oesophagus, stomach, small intestine and large intestine as against the control group one which was fed with pellet and water only with normal cells across the various parts of the gastrointestinal tract studied. Ulcers arise as a result of excessive stomach acid production or a decline in gastric mucosal defensive mechanisms. It is well known that NSAIDs, such as aspirin, decrease prostaglandin synthesis, which increases vulnerability to mucosal damage and causes

stomach ulcers (Atay *et al*, 2000). Geetha *et al*, (2011), report that some anti-ulcerogenic and ulcer-healing medications are known to reduce acid secretion in the stomach mucosa. Aspirin-induced ulcers occur as a result of mucosal damage caused by interfering with prostaglandin synthesis, increased acid secretion, and back diffusion of H⁺ ions, resulting in the breakdown of mucosal barriers (Geetha *et al*, 2011). In a study by wan-tong *et al*, (2021), aspirin breaks down the stomach mucosal barrier, which allows gastric acid to directly break down mucosal epithelial cells, resulting in inflammation, bleeding, and gastric ulcers. This persistent inflammation is what causes aspirin-induced gastric damage.

Group 4 of this study which was studied to assay for the counter effect activity of the extract against aspirin, feed with the 1000mg/kg of extract and 20mg/kg of aspirin simultaneously showed normal cell in the oesophagus, stomach, small intestine and large intestine, this maybe due to the phytochemicals contained in the extract such as saponins and flavonoids. *P.biglobosa* has been shown to have anti-inflammatory properties (Kouadio *et al*, 2000). *P. biglobosa* is thought to exert its protective benefits by inhibiting the lipoxygenase and cyclooxygenase pathways (Nwaehujor *et al*, 2010). This prevents the release of pro-inflammatory cytokines and stimulates the production of anti-inflammatory cytokines (Silva *et al*, 2013) while also increasing membrane stability (Ukwuani *et al*, 2015). In a study by Antoine *et al*, (2022), which studied the effect of aqueous extract of *P.biglobosa* on antiepileptogenic, anti-amnesic, and anxiolytic-like effects in mice via mechanisms involving antioxidant and anti-inflammatory pathways. The pro-inflammatory cytokines were dramatically reduced after administration of the aqueous extract of fermented seed of *P.biglobosa*, indicating anti-inflammatory efficacy. This could be as a result of the presence of secondary metabolites, studies on the phytochemical screening of *P.biglobosa* revealed the presence of phytoconstituents like anthraquinones, tannins, flavonoids, terpenes,

saponins, phenols, and steroids, which may be related to *P. biglobosa*'s capacity to heal wounds (Builders *et al*, 2019).

Saponins are a heterogeneous group of naturally occurring surface active glycosides that are mostly produced by plants, but also by lower marine creatures and some microorganisms (Francis *et al*, 2002), while Flavonoids are polyphenolic chemicals found in plants that have a variety of biological effects (Permender *et al*, 2009). Triterpenoid or steroid aglycone moiety and complicated oligosaccharide substituent make up their structure. Saponins have amphiphilic or surfactant qualities due to the hydrophilic nature of the sugar component and the lipophilic nature of the aglycone part, which result in their capacity to create stable aqueous foams as well as complexes with membrane steroids and lipid molecules (Hostettmann and Marston, 1995). Because they can directly affect pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-) and interleukin-6 (IL-6), inhibit the activity of macrophages, and act on the arachidonic acid (AA) pathway to reduce cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) activity, steroidal saponins are effective therapeutic options for treating inflammatory diseases. The suppression of eicosanoid producing enzymes such as phospholipase A2, cyclooxygenases, and lipoxygenases reduces the amounts of prostanoids and leukotrienes, which is a key mechanism for anti-inflammatory effect of flavonoids. Other pathways include histamine release inhibition, phosphodiesterase inhibition, protein kinase activation, and transcriptase activation (Permender *et al*, 2009).

Group five of this study were administered 1200mg/kg of the extract for a week and 20mg/kg of aspirin the following week, was used to observe the preventive effect of the extract. Group six was administered 20mg/kg of aspirin for a week and 1500mg/kg of the extract the following week was used to assay for the reversal effect of the extract. Both group five and six had various

parts of the gastrointestinal tract still inflamed, which could be dose dependent or the duration of administration related. In a research by Gabriel et al, 2021, which studied the effect of aqueous seed extracts of *P.biglobosa* on the histology and glycogen profile of the epididymis of wistar rats, the results obtained in the study suggested that aqueous seed extract of *Parkia biglobosa* may have an adverse effect on the epididymis when treated at high dose.

5.2 CONCLUSION

From this research it may be inferred that *Parkia biglobosa* fermented seed extract may have a counteracting effect against aspirin. This is very beneficial because it could reduce the number of mortality as a result of gastric ulcer and bleeding especially for those on long term use of aspirin.

5.3 RECOMMENDATION

Parkia biglobosa may have possible ameliorative properties in the gastrointestinal tract of the male wistar albino rat with histological alterations. It is important that additional and focus research be carried out to ascertain these effects in relation to aspirin induced gastrointestinal tract injury.

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APPENDIX I

The instrument used for this research is as follows:

1. Animal House: during the time of feeding

- a. Metal cage
- b. Feed guinea food
- c. Feeding water bottle
- d. Digital thermometer
- e. ISOL disinfectant
- f. Feeding flat plate
- g. Indian ink and plate
- h. weight balance

2. For Sacrificing

- a. Chloroform
- b. Plastic container sterile with a cover
- c. Formalin
- d. Dissecting set
- e. Cotton wool

Sterile containers

- g. Sterile lancet
- h. Hand gloves

3. Histology Laboratory

- a. Tissue basket
- b. Rotary type microtome

- c. Hot plate
- d. Metal pencil
- e. L-shaped mould
- f. Scrape blade
- g. slides and cover-slip
- h. Automatic tissue processor
- i. Water bath
- j. Spatula
- k. Molten basket
- l. Binocular microscope
- m. Block holder
- n. Stain: haematoxylin and eosin
- o. DPX (dibutylphthalatepolyesterene xylene), xylene, alcohol and water.

APPENDIX II

- A. Melted paraffin wax was poured into the mold.
- B. Warm blunt-nosed forceps were used to transfer tissues from the paraffin bath to the mould.
- C. Tissues were positioned till lying in the proper plane using warmed forceps.
- D. The corresponding labels from the paraffin bath were taken off and placed next to the tissues on the side of the mould.
- E. Air was blown on the surface until a thin film of wax has solidified.
- F. The mold was moved into a bowl of cold water and submerged there until the wax hardened.
- G. After embedding, the block is allowed to freeze on ice for a few hours before being sectioned.
- H. Trimming and sectioning were done using a Hertz rotary microtome (Cambridge model), with the block clamp set so that sections 3-5 um were formed in a ribbon-like fashion. These sections were then floated in a water bath to flatten by mild heat.
- I. To ensure that the sections were properly dried before staining by placing them on a hot plate, the section or short ribbon was chosen using a clean, grease-free slide. Slides were then stained using the Haematoxylin and Eosin technique.

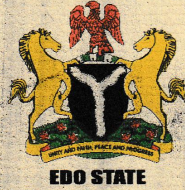
APPENDIX III

STAINING TECHNIQUE

- A. The sections were dewaxed in two changes of xylene for 2 minutes each.
- B. Sections were taken through descending grades of alcohol. From absolute alcohol for 2 minutes, to 90% alcohol for 1 minutes, 70% alcohol for 1 minutes.
- C. The slides were washed in running tap water for one minute.
- D. Tissue sections were stained in hematoxylin for 10 minutes
- E. The tissue sections rinsed in distilled water for 30 seconds.
- F. Sections were differentiated in 1% acid alcohol for 15 seconds
- G. Sections were rinsed in distilled water for 5 minutes.
- H. Sections were counter stained with 1% eosin for 5 minute.
- I. Sections were washed in running tap water for 30 seconds.
- J. Sections were dehydrated by passing through ascending grades of alcohol (70%, 90%, 100%) for 1 minute each.
- K. Sections were cleared in two changes of xylene for 2 minutes each.
- L. Sections were mounted with DPX and viewed microscopically using objective lenses.

APPENDIX IV

ETHICAL APPROVAL



MINISTRY OF AGRICULTURE AND FOOD SECURITY

V.1041/89

23rd January, 2023

ANOZIE, CHINWENDU AMARACHI

Dept. of Medical Lab. Science

College of Basic Medical Sciences

University of Benin.

RE: YOUR APPLICATION TITLED: EFFECTS OF Parkia biglobosa FERMENTED SEED EXTRACT AFTER Aspirin- Induced Gastrointestinal Tract Injuries in Animal Model : REQUEST FOR ETHICAL CLEARANCE.

I write to acknowledge the receipt of your letter Ref: 2023/01/RL 007 dated 12th, January 2023 on the above subject matter and to convey our interest in your research work which will further broaden the horizon of usefulness of animals in the field of Science and Technology.

In as much as your experiment is limited to working with organ and tissue samples from these animal models, our ethical consideration is to ensure that a standard procedure is adhered to accordingly.

In view of the above, i wish to convey an official approval and recommend that you contact Dr, Daniel Asemota of Faculty of Veterinary Medicine, University of Benin to assist.

The Research Ethics Review Committee wishes you all the best with this research undertaking and would be pleased to receive a copy of the summary of your research study.

Kind regards.

I write to acknowledge the receipt of your letter Ref: 2023/01/RL 007 dated 12th, January 2023 on the above subject matter and to convey our interest in your research work which will further broaden the horizon of usefulness of animals in the field of Science and Technology.

[Handwritten signature]

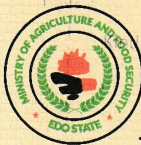
Dr. T. A. Haruna

(MAFSVS Department Ethics Committee)

In view of the above, i wish to convey an official approval and recommend that you contact Dr, Daniel Asemota of Faculty of Veterinary Medicine, University of Benin to assist.

The Research Ethics Review Committee wishes you all the best with this research undertaking and would be pleased to receive a copy of the summary of your research study.

Kind regards.



Ezoti Street, P.M.B. 1060, Benin City, Edo State, Nigeria.
www.edostate.gov.ng/agriculture

APPENDIX V

Soxhlet Extractor



APPENDIX VI

Parkia biglobosa

