THE EFFECTS OF AQUEOUS EXTRACT OF MISTLETOE *(Viscum album)* ON LEAD ACETATE-INDUCED GASTRIC DAMAGE IN ADULT WISTAR RATS

BY

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SCHOOL OF BASIC MEDICAL SCIENCES

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

SUPERVISOR: DR. ENDURANCE IMAFIDON

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc.) IN ANATOMY.

JANUARY, 2023.

CERTIFICATION

This is to hereby certify that this research was carried out by **EGBON ADEDOJA** with matriculation number **BMS1701931** in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. In partial fulfilment of the requirement for the award of Bachelor of Science Degree (B.Sc.) in Anatomy.

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(HEAD OF DEPRTMENT)

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EXTERNAL EXAMINER **DATE**

DEDICATION

This work is dedicated to Almighty God, for His love, grace and faithfulness. It is also dedicated to my Dad for his love and support throughout the course of this programme. To my Big mummy for her care, support and love. Lastly to my siblings Olamide Egbon and Joshua Egbon my best persons for always supporting, encouraging and believing in me.

ACKNOWLEDGEMENT

My sincere gratitude goes to God Almighty that made this research project a reality. I express my profound gratitude to my Dad, Mr Monday Egbon for your unending support, thank you for doing everything possible to make this journey an easy one. God bless and keep you Daddy. To my family, Olamide Egbon, Joshua Egbon and My Big mummy Mrs Bodunrin Akhimien. Thank you all for your ceaseless support in prayers, encouragements, love and provisions.

I would like to acknowledge my supervisor Dr Endurance Imafidon for his guidance and patience during the course of this project. I also appreciate Dr. Silvanus Olu Innih , who is also the Head of department of Anatomy for his guidance, patience and words of advice. To my other lecturers Dr.Vitalis, Prof. Eze for their advice and words of wisdom.

I appreciate Grace mum,Mrs Angela Egechi for her spiritual guidance, prayers and checkups all through this journey, God bless you abundantly mummy.

To my friend and sister Double G, thank you for everything, to your rising there shall be no end, God bless you.

To my lovely friends; Godsgrace, Favour, Amenze, Cherish, Gladys, Blessing and inioluwa. Thank you so much for being part of this journey with me. You all are wonderful.

I also want to appreciate DOS John for his immerse assistance during the course of this project, God richly bless you. And to Mr Sam, thank you sir.

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ABSTRACT

Consuming toxic chemicals and substances can induce gastric damage to the stomach, which can result in serious medical disorders, cancer, and even death. Traditional herbs are being studied and processed into contemporary medications that are used to treat a variety of illnesses and medical issues. Mistletoe extracts have a long history of usage as secondary treatments for a variety of illnesses. Additionally, they include antioxidants such flavonoids, which have effects both in vitro and in vivo, and they have also been documented to exert specific pharmacological actions (such as cytotoxic and immunomodulatory). This study was carried out to investigate the effects of aqueous extract of mistletoe on lead acetate-induced gastric damage in adult Wistar rats. Thirty (30) Wistar rats weighing between 150g and 250g were grouped into six; Group A, B, C, D, E and F. Each groups was made of up 5 rats and the rats were fed with grower mash feed (Primer Feed mill, Nigeria) and had free access to water throughout the entire period of study. Group A served as control. Group B rats were given 10mg/kg of lead acetate and 200mg/kg extract of Mistletoe (low dose). Group C rats were given 10mg/kg of lead acetate and 400mg/kg extract of Mistletoe (intermediate dose). Group D were given 10mg/kg of lead acetate and 800mg/kg extract of Mistletoe (high dose). Group E were given 10mg/kg of lead acetate only. Group F were given 10mg/kg of lead acetate and standard drug 500mg/kg (Omeprazole). After receiving the treatment for 28 days, the rats were sacrificed. Choloform was used to render the rats unconscious. The stomach was removed, weighed, and placed in 10% formal-saline to prevent autolysis before being sent to the University of Benin Teaching Hospital (UBTH) histopathology department for tissue processing and histological evaluation. The results from this research shows that there was significant increase ($P<0.05$) of body weight in Group A, B, C, D and F when the initial body weight were compared to the final body weights. There was no significant difference ($P>0.5$) of gastrosomatic index across all the groups. There was no significant difference ($P>0.05$) of gastric weight across all the groups. The *Viscum album* extract had a dose dependent ameliorative, therapeutic and protective effect on the gastric damage caused by lead acetate on the stomach as revealed and seen in the histology results.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Today, due to their potential medical benefits, plants and plant-based products are in high demand (Koparde, 2019). Traditional herbs and plants have been used for many years to treat many diseases. Examples include ginger, which heals chronic dyspepsia and also works to combat infections, and turmeric, which has shown promise in treating joint arthritis (Debij, 2019).

In the current world, traditional plants are being researched and transformed into pharmaceuticals that we use to cure various illnesses (Singh, 2020).

The parasitic flowering plant *Viscum Album*, also known as mistletoe, belongs to the Loranthaceae, Misodendraceae, and Santalaceae plant families. It is a tiny evergreen shrub that somewhat parasitizes other species. Instead of growing roots in the earth, mistletoe sends out structures resembling roots into tree branches, where it absorbs nutrients and water (Patil, 2022).

Mistletoe grows on trees, and it favours hosts with plenty of sunlight. The National Cancer Institute (NCI) reports that mistletoe has been used for many years to cure a variety of ailments, including rheumatism, hypertension, migraines, menopausal symptoms, and epilepsy (Sacheer, 2013).The mistletoe has green branches, oval-shaped leaves, and tiny, sticky, whitish berries, while the dwarf mistletoe is smaller and has leprose yellowish or orange leaves (Amanda, 2014). Afomo, Awuruse, and Bokondoro are the names for mistletoe in Nigerian languages (Sade, 2016).

One of the most popular forms of lead is lead acetate, a white, crystalline molecule with a sweetish taste that is often known as "sugar of lead." Lead acetate is soluble in water. It dissolves in water and is extremely poisonous. Lead acetate creates the trihydrate Pb(ch3coo)2 when there is water present. (Mohammed and James, 1964). It is utilised in pesticides, waterproofing, antifouling coatings, and the gold cyanidation process.

The intention of this project is to determine the impact of an aqueous extract of *Viscum album* on the histology and morphology of the adult Wistar rat stomach. This experiment will help people understand the effect of *Viscum album* on the stomach.

1.2 AIM OF THE STUDY

The aim of this study is to investigate the effects of aqueous extract of Mistletoe on lead acetate-induced gastric damage in adult Wistar rats.

1.3 SPECIFIC OBJECTIVES OF THE STUDY

The specific objectives of this study were to investigate the effect of aqueous extract of mistletoe on;

- Body weight of adult Wistar rats.
- Organ (stomach) weight of adult Wistar rats
- Organosomatic index of the adult Wistar rats
- Stomach histology of adult Wistar rats

1.4 SIGNIFICANCE OF THE STUDY

Humans are exposed to a variety of harmful chemicals and compounds, such as lead acetate and mercury chloride. One of the most used chemicals is lead acetate, which is used in antifouling paints, waterproofing, pesticides, the gold cyanidation process, as well as other significant sectors. Since ancient times, medicinal plants have been employed in traditional medicine (Awuchi, 2019). There are many phytochemicals that have been discovered as having potential or proven activity, but since a single plant has a vast variety of phytochemicals, it is unclear how utilising a full plant as medication may affect you (Awuchi, 2019). This study aims to determine whether mistletoe's *(Viscum album*) aqueous extract can reverse the damage that lead acetate causes to adult Wistar rats' stomachs.

The results of this study will shed insight on the impact of aqueous mistletoe extract on experimentally caused stomach injury in adult Wistar rats. They will also add to the existing knowledge already known about the topic, which will serve as the basis for future research.

1.5 STATEMENT OF PROBLEM

The Stomach plays a vital role in the storage and breakdowm of food and substances that enters the body. Medicinal plants are used in traditional medicine practices since pre-historic times (Ahn, 2017). Numerous phytochemicals with potential or established activity have been identified but since a single plant contains widely diverse phytochemicals, the effect of using a whole plant and medicine is uncertain (Ahn, 2017). This research was proposed to investigate the effect of aqueous extract of *Viscum album* on lead acetate-induced gastric damage in adult Wistar rat.

CHAPTER TWO

LITERATURE REVIEW

2.1 PLANT OF STUDY

Before the development of modern medicine, man has used plants and herbs to treat illnesses and mend wounds for ages. The usage of medicinal plants has garnered interest recently, and scientific research is starting to shed light on some of the healing phenomena connected to age-old cures. Some plants have medicinal properties and were employed in herbalism. These medicinal plants have a bright future since they are a rich source of components and are utilised as raw materials in the production of pharmaceuticals. Antibiotics, blood thinners, anti-malarial drugs, and opioids all contain plant-based components. (Rasool, 2012).

FIGURE 1: Mistletoe (*Viscum album*) growing on tree (Marcus Schneck 2019)

In all climates, a wide variety of tree species are being impacted by biotic disturbances, and their presence is having an impact on and contributing to rising tree mortality rates. A large family of parasitic plants known as mistletoe has enduring connections with a wide variety of host tree species. As a result of climate change, trees are more vulnerable to mistletoe infection, which raises the rate of forest mortality. Within the scientific community, there is disagreement regarding how much mistletoe is actually present in individual trees and forest stands. Mistletoe can be viewed as a threat by forest managers who are worried about the health of their stands because it causes trees to produce less. However, because it fosters nutrient cycling and wildlife habitat, ecologists might view mistletoe as a friend (Anne Griebel *et al.,* 2017).

2.1.1 SPREAD OF MISTLETOE

Birds that consume the berries that contain the sticky seeds then eat the sticky seeds they produce in the tops of larger trees where they like to perch, which is how mistletoe (Figure 1) is distributed. Pruning affected branches or removing mistletoe stems from the branches or trunks on a regular basis will help control the infestation in solitary fruit or shade trees (Reid, 1989).

2.1.2 HOST/PLANT RELATIONSHIP

In the tropics, mistletoe is regarded as a parasitic plant that grows untamed on the branches or trunks of commercial trees. In Nigeria, mistletoe grows untamed on a variety of commercial trees, which are typically felled and killed. This occurs as a result of the harm they do to their host trees, which results in significant financial loss. Through adventitious roots that pierce through the tissues of their host and negatively impact the growth and fruiting abilities of the host tree, mistletoes anchor themselves to their host. While mistletoe can manufacture carbohydrates through photosynthesis, it only demands water and minerals from its host (Ishiwu et al., 2013).

The xylem water potential of the host is most adverse when host photosynthesis is at its peak. The mistletoe needs a greater negative water potential than the host in order to maintain a flux gradient and prevent stomatal closure and withering. Succulent leaves improve water retention and enable mistletoe to hydrate independently of its hosts (Glatzel and Geils, 2009). The stomatal control system of the host may be disrupted by mistletoe infections, leading to early and oscillatory stomatal closure and a reduction in the photosynthetic gain of the host. Mistletoes depend on the connection with the host through the haustorium for the essentially one-way flow of photosynthates and nutrients from host to parasite because they lack the active uptake of minerals found in a typical plant root system. Mistletoes can resist mineral excess or shortage by a variety of mechanisms, including tolerance, succulence, and quick leaf turnover. Every setting and time period presents a different relationship between the host state and mistletoe performance. The host may occasionally outgrow the mistletoe, but if the host is in a good mood, the mistletoe can grow more quickly (Glatzel and Geils, 2009).

2.1.3 CONSTITUENTS OF MISTLETOE EXTRACT

The extract of the mistletoe plant's stems, roots, leaves, buds, flowers, and berries includes a variety of substances, including:

- There are about 600 different types of proteins; the precise protein range varies depending on the host tree on which the mistletoe grew.
- The sugar-containing mistletoe lectins, of which there are various types and various combinations are present in the total extract, are the most significant mistletoe-specific proteins (Franz *et al.*,1981).

- Various viscotoxins, which are among the essential pharmaceutically potent elements of mistletoe together with lectins (Winterfield and Bijl, 1949).
- More than seven distinct resin compounds (pentacyclictriterpenes) that have the ability to suppress the growth of tumours, but which are only present in very small amounts in commercially available preparations for aqueous injection. This is due to their fat-solubility.
- There are over a thousand distinct enzymes.
- Extreme DNA concentration (deoxyribonucleic acid, the chemical building blocks of genetic material).
- The mistletoe contains sulphur-rich chemicals that have thiol (e.g. glutathione) concentrations that are up to a thousand times greater than those of other plants.
- Various lipids (fats), such as membrane lipids, waxes, and triglycerides.
- Different flavonoids (yellow, red and blue plant pigments) (Kinele and kiene,2003).
- Potassium and phosphate. (Marion *et al.,* 2020).

2.1.4 TRADITIONAL/ETHNOBOTANICAL USES

In Europe, medicines made from mistletoe have been used medicinally for ages to treat conditions like arthritis, hypertension, infertility, and epilepsy. Because mistletoe is parasitic and fatal to its host, like cancer, Rudolf Steiner, the Austrian founder of the anthroposophical movement, hypothesised that it might be utilised to treat cancer in 1921 (Singh *et al.*, 2016). To put this theory into practise, Swiss and German clinics were established, and they still make use of a mistletoe preparation that has been fermented with a strain of Lactobacillus for three days. Mistletoe extracts include a number of poisonous proteins, many of which are lectins, or proteins that may bind to particular sugars. To treat hypertension, 10 g/day of crude mistletoe fruit or herb is used to brew a tea. Numerous proprietary extracts with trace amounts of mistletoe lectin-I (ML-I) are used as adjuvant cancer treatments. These extracts are often administered by intravenous or subcutaneous injection at doses ranging from 0.1 to 30 mg many times per week. Depending on the patient's overall health and how they react to the injection, mistletoe preparations made using anthroposophical procedures are administered in dosages that gradually increase. It has been reported to be used in paediatric patients. Adults in good health have had their pharmacokinetics assessed (Drugs.com, 2018).

2.1.5 TAXONOMY/ CLASSIFICATION OF MISTLETOE

Preferred Scientific Name: *Viscum album*

Preferred Common Name: Mistletoe

Taxonomic Tree

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledona

2.1.6 HEALTH BENEFITS OF MISTLETOE

- **Anxiety/Stress Relief**

  Anxiety that is persistent and ongoing negatively affects day-to-day functioning. There may be calming effects of mistletoe (Beshay, 2018). According to studies, mistletoe may help cancer patients feel less depressed and anxious (Kienle and Kiene ,2010).

Your physical and mental health depend on getting a good night's sleep, which can also significantly aid in managing anxiety. Mistletoe has a long history of use as a herbal sleep aid. These herb's chemical constituents might encourage the release of neurotransmitters like dopamine to help sound sleep (Xie W *et al.*, 2017).

- **Cancer Care**

 Mistletoe is one of the most thoroughly studied complementary treatments for cancer patients, according to the National Cancer Institute research, mistletoe may boost the immune system's ability to fight cancer. In an experimental laboratory context, mistletoe suppresses cancer cell multiplication (spread) and even kills pre-existing cancer cells, demonstrating that mistletoe extracts have anti-cancer effect. Mistletoe may help cancer patients live better lives, according to research. (Loef and Walach, 2020) Additionally, it might raise blood levels or perhaps lessen tumour growth. (Klopp *et al*., 2001) According to some studies, mistletoe helps lessen the side effects of chemotherapy, including nausea, lack of appetite, discomfort, exhaustion, and depressive or anxious symptoms.

- **Cardiovascular Health**

 Vascular disorders including heart disease and kidney disease can be brought on by atherosclerosis, a condition linked to persistent hypertension and a number of other conditions. High blood pressure, coronary heart disease, and stroke may all be prevented with mistletoe use. (Karagoz *et al*., 2016), According to research, mistletoe enhances circulation and protects the heart and blood vessels by acting as an antioxidant. Mistletoe may also assist in controlling heart rate (Orhan *et al.*, 2005).

- **Immune System and Respiratory Health**

  Mistletoe may strengthen and maintain the immune system, aiding in the prevention of infections thanks to its antioxidant, antibacterial, and antiviral qualities. Mistletoe has been shown to relieve respiratory disorders including asthma in animal tests, despite the fact that there is little human study (Fikenzer and Laufs, 2020).

- **Anti-inflammatory**

  As a result of the plant's anti-inflammatory and antioxidant characteristics, it may be used to treat inflammatory chronic illnesses like arthritis. The body responds to infection, damage, and disease by inflaming, but many autoimmune disorders also exhibit misdirected inflammation. (Khan *et al.*, 2016) Mistletoe tinctures have been used for hundreds of years by people to help lessen inflammation both within and outside of the body. According to research, mistletoe extract may also help with digestive health by reducing inflammation-related gastrointestinal problems (Patil *et al*., 2011).

- **Diabetes Control:**

  For centuries, mistletoe has been used to help regulate blood sugar levels. Research suggests that mistletoe may be used to treat diabetes (Gray and Flatt, 1999). Mistletoe lowers blood glucose levels and increases insulin synthesis in pancreatic cells, according to studies on animal models. Mistletoe extract may shield liver cells from damage caused by free radicals, according to one study. To fully comprehend mistletoe's function in maintaining human glucose levels in balance, more research is required (Onunogbo, Ohaeri and Eleazu, 2013).

2.1.7 POSSIBLE SIDE EFFECTS

Mistletoe rarely causes negative effects when administered in the prescribed dosages. Generally speaking, when the dosage is too high, adverse symptoms such nausea, vomiting, diarrhea, low blood pressure, or dizziness can occur.

Mistletoe injections, such as those given to cancer patients as a natural supplement, may result in discomfort and inflammation at the injection site, but no serious side effects have been linked to them (Huber *et al.,* 2017).

Children, women who are pregnant, or those who are nursing should not use mistletoe. Before consuming mistletoe, it is crucial to see a healthcare professional, especially if you are using any prescription drugs.

Many medications, including anticoagulants, antidepressants, and drugs for treating heart disease or high blood pressure, may interact with mistletoe.

2.2 LEAD ACETATE

Lead (II) acetate, also referred to as lead acetate, is a chemical compound that has a taste that is slightly sweet and is a crystalline solid that ranges in colour from white to grey. It is hazardous, just like many other lead compounds. Glycerin and water both make lead acetate soluble (NCBI 2022).

2.2.1 ELEMENT PROPERTIES

In addition to being a fixative for some dyes, lead acetate is a reagent used to create additional lead compounds. It is the main active component of various types of hair colouring colours in low quantities. In addition to being a drier in paints and varnishes, lead(II) acetate is also employed as a mordant in the printing and dyeing of textiles. Historically, it was employed in wines and other foods as a sweetener and preservative.

Table 1: Showing the General Properties of Lead acetate.

Chemical Formula	$Pb(C_2H_3O_2)_2$
Molar Mass	325.29 g/mol (anhydrous) 379.33g/mol (trihydrate)
Appearance	White powder or colourless, efflorescent crystals
Odor	Slightly acetic
Density	3.25 g/cm^3 (20 °C, anhydrous) 2.55 g/cm^3 (trihydrate) 1.69 g/cm^3 (decahydrate)
Melting Point	280 °C (536 °F; 553 K) (anhydrous) 75 °C (167 °F; 348 K) (trihydrate) decomposes at ≥ 200 °C 22 °C (72 °F; 295 K) (decahydrate)
Boiling point	Decomposes
Solubility in water	Anhydrous: 102.75 g/100 g (66.1 °C) Trihydrate 74.75 g/100 g (15 °C) 214.95 g/100 g (66.1 °C)

2.2.2 USES OF LEAD

- **Sweetener**

  Lead(II) acetate, like other lead(II) salts, has a sweet flavour, which has historically led to its use as a sugar substitute in both wines and dishes (Archibald, 2020). The ancient Romans, who had few alternatives to honey as a sweetener, would boil must (grape juice) in lead pots to create defrutum, a reduced-sugar syrup that was then concentrated again to create saps. This syrup was employed to preserve and sweeten fruit as well as to sweeten wine. It's probable that lead poisoning in those who consumed the syrup was brought on by lead (II) acetate or other lead compounds seeping into it. Due to its known toxicity, lead acetate is no longer utilized in the manufacturing of sweeteners.

- **Cosmetics**

  In 1973, Gunn and Fenja White lead and lead (II) acetate have both been used in cosmetics throughout history. It was still utilised until recently in the USA in hair colouring products for males like Grecian Formula. Grecian Formula's constituents as of July 2018 are water, isopropyl alcohol, triethanolamine, bismuth citrate, sodium thiosulfate, aroma, and panthenol. Lead acetate was only recently removed from the product by the manufacturer (Howard *et al.,*1997).

- **Medical Uses**

  A traditional home treatment for aching nipples was lead (II) acetate solution. It has been used to treat poison ivy and was once employed as an astringent in contemporary medicine in the form of Goulard's Extract.

- **Industrial Uses**

  Hydrogen sulphide, an extremely dangerous gas, is detected using lead (II) acetate paper. On the moistened test paper, the gas combines with lead (II) acetate to produce a lead (II) sulphide precipitate, which is grey in colour. During the middle Ages, it was also employed in the production of slow matches.

In order to make "boiled" linseed oil, sugar of lead was advised to be added. The lead and heat caused the oil to cure more quickly than raw linseed oil.

2.3 ORGAN OF STUDY: STOMACH

Food is digested by the stomach, a J-shaped organ. Acids and enzymes, which are chemicals that catalyse chemical reactions, are produced (digestive juices). Food is broken down by this concoction of digestive juices and enzymes so that it may move on to your small intestine. A component of the gastrointestinal (GI) tract is the stomach. The GI tract is a protracted tube that originates in the mouth and terminates in the anus, where stools and faeces leave the body.

The digestive system's main component is the GI tract. Food must be digested in the stomach before being sent to the small intestine. The upper abdomen on the left side of the body is where the stomach is located. The esophageal sphincter, a valve, is connected to the top of the stomach (a muscle at the end of the esophagus). The small intestine is joined to the stomach at its base. Everybody has a different-sized stomach. When the stomach is full, it swells; when empty, it contracts. As a result, the stomach's size varies according to how recently and how much food is consumed.

2.3.1 ANATOMY OF THE STOMACH

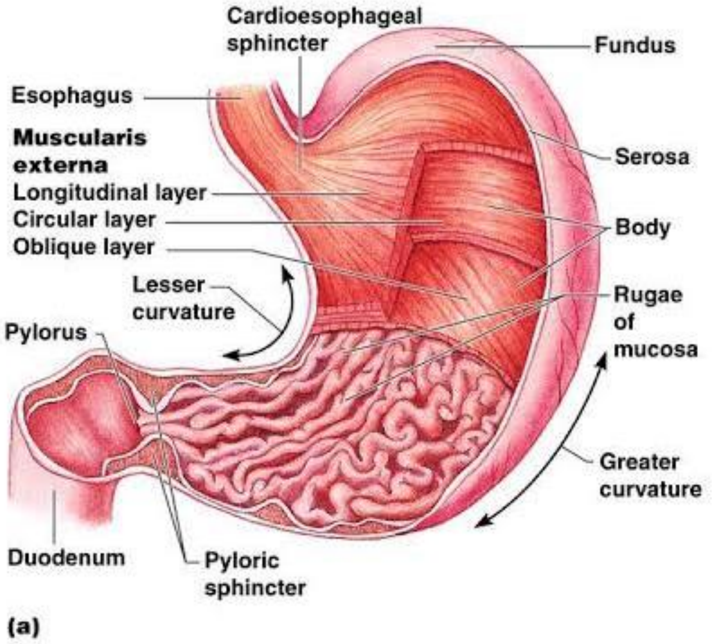

Figure 2: Gross Anatomy of the Stomach (Research Gate 2012).

2.3.2 PARTS AND CURVATURE OF STOMACH

The stomach's shape varies greatly from person to person and is dynamic (changing as it performs) (Moore *et al.*, 2018).There are four distinct stomach regions and two curves.

The cardial orifice, the trumpet-shaped entry of the oesophagus into the stomach, is surrounded by the short cardia.

• The fundus is the expanded upper portion of the stomach that is connected to the left dome of the diaphragm and is restricted inferiorly by the horizontal plane of the cardial orifice. Normally, the superior portion of the fundus is level with the left fifth intercostal gap. The cardial notch is situated between the fundus and the oesophagus.

Gas, liquid, food, or any combination of these may cause the fundus to widen (particularly while the patient is standing upright).

• Between the fundus and the pyloric antrum is the stomach's main portion, known as the body. The fundus and body are frequently used interchangeably by histologists and pathologists; as a result, the mucosa of the fundus and body is made up of fundic glands (Moore *et al.,* 2018).

• The distal funnel-shaped section of the stomach is called the pyloric part; its wide part, the pyloric antrum, enters the pyloric canal, which is its narrow part. The pylorus, also known as the distal sphincteric region, is a thickening of the circular layer of smooth muscle that regulates the passage of stomach contents into the duodenum through the pyloric opening.

• The stomach's shorter concave border is formed by the lesser curvature, and the angular incisure (notch), which is located about two thirds of the way along the lesser curvature and roughly marks the intersection of the body and pyloric parts of the stomach, has a sharp indentation (Moore *et al.,* 2018).

• The longer convex border of the stomach is formed by the greater curvature.

2.3.3 INTERIOR OF STOMACH

The majority of the longitudinal gastric folds (rugae) are formed as the stomach mucosa contracts. These are more pronounced at the greater curvature and near the pyloric portion. During swallowing, a gastric canal (furrow) arises between the longitudinal gastric folds along the lesser curvature. When the stomach is mostly empty, saliva, small amounts of chewed food, and other fluids flow via the gastric canal to the pyloric canal (Moore *et al.,* 2018).

2.3.4 STRUCTURES OF THE HUMAN STOMACH

In the stomach, food begins to be digested and absorbed, though most substances other than water, alcohol, and some medications can also be absorbed. The muscular pyloric sphincter contracts to retain swallowed food inside the expanding, muscular stomach. Food can linger in the stomach for at least two hours. Gastric juice and the contraction of the three layers of smooth muscles in the muscular exterior layer break down food chemically and mechanically, respectively. At the conclusion of this procedure, the food fragments are known as chyme (Moore *et al.*, 2018).

Gastric mucosal glands release gastric juice, which is made up of hydrochloric acid, mucus, and the proteolytic enzymes pepsin and lipase (which breaks down fats).

The lining of the stomach is thrust up into folds known as rugae when it is empty and not bloated. These folds go flat after eating, allowing the stomach to expand significantly.

An exterior longitudinal layer, a middle circular layer, and an interior oblique layer make up the three layers of muscle that make up the stomach. There are four layers in the interior coveringthe muscularis, serosa, submucosa, and mucosa. Gastric glands, which include cells that create digesting enzymes, hydrochloric acid, and mucus, are incredibly numerous and packed closely together in the mucosa.

2.3.5 DEVELOPMENT OF THE STOMACH

The distal portion of the foregut has a beautiful structure at first. The location of the stomach primordium is indicated by a modest dilatation around the middle of the fourth week. It initially manifests as an expansion caused by fusion in the caudal or distal portion of the foregut and is positioned in the median plane. The primordium stack rapidly grows and ventrodorsally broadens. Over the course of the following two weeks, the stomach's dorsal border expands more quickly than its ventral border, defining the stomach's larger curvature.

Rotation of the Stomach

The stomach slowly rotates 90 degrees clockwise (as seen from the cranial end) around its longitudinal axis as it grows and takes on its ultimate shape. The effects of rotation on stomach are

1. The dosal border, which has greater curvature, shifts to the left, while the ventral border, which has less curvature, shifts to the right.
2. The initial right side turns into the dorsal surface, while the initial left side becomes the ventral surface.
3. The stomach's cranial and caudal ends are in the median plane prior to rotation. The stomach expands and riates, moving its caudal region to the right and superiorly, and its cranial region to the left and slightly inferior.
4. The stomach rotates into its final position, with its long axis almost parallel to the body's long axis. The left vagus nerve supplies the adult stomach's front wall, while the right vagus nerve innervates its posterior wall. This is due to the rotation and expansion of the stomach.

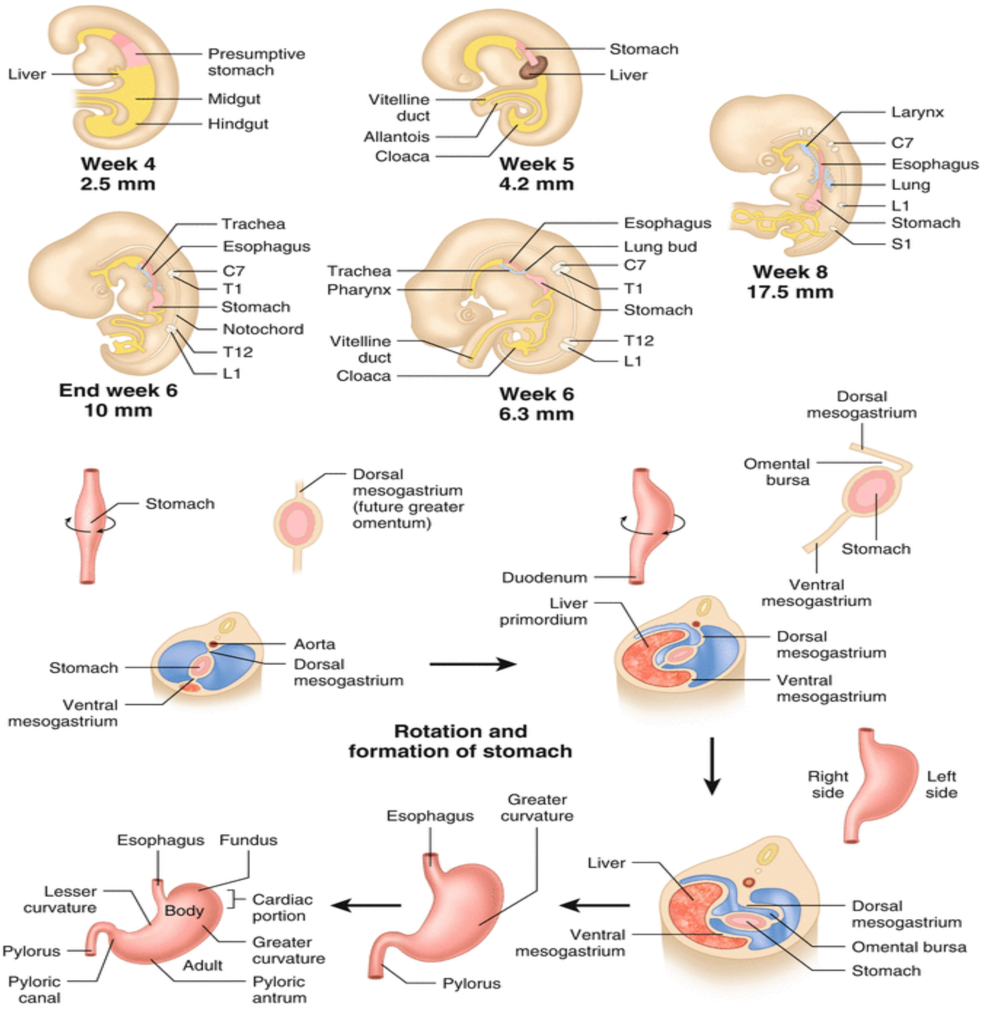

Figure 3: Embryology of the Stomach (Springer Berlin 2017).

Mesenteric of the Stomach

The dorsal mesentery, or the primordial dorsal mesogastrium, suspends the stomach from the dorsal wall of the abdominal cavity. When the stomach rotates and the omental bursa, or smaller sac of peritoneum, forms, this mesentery, which was initially in the median plane, is carried to the left. Stomach and the primordial ventral mesogastrium are connected. The duodenum is connected to the liver and the ventral abdominal wall by the mesogastrium as well.

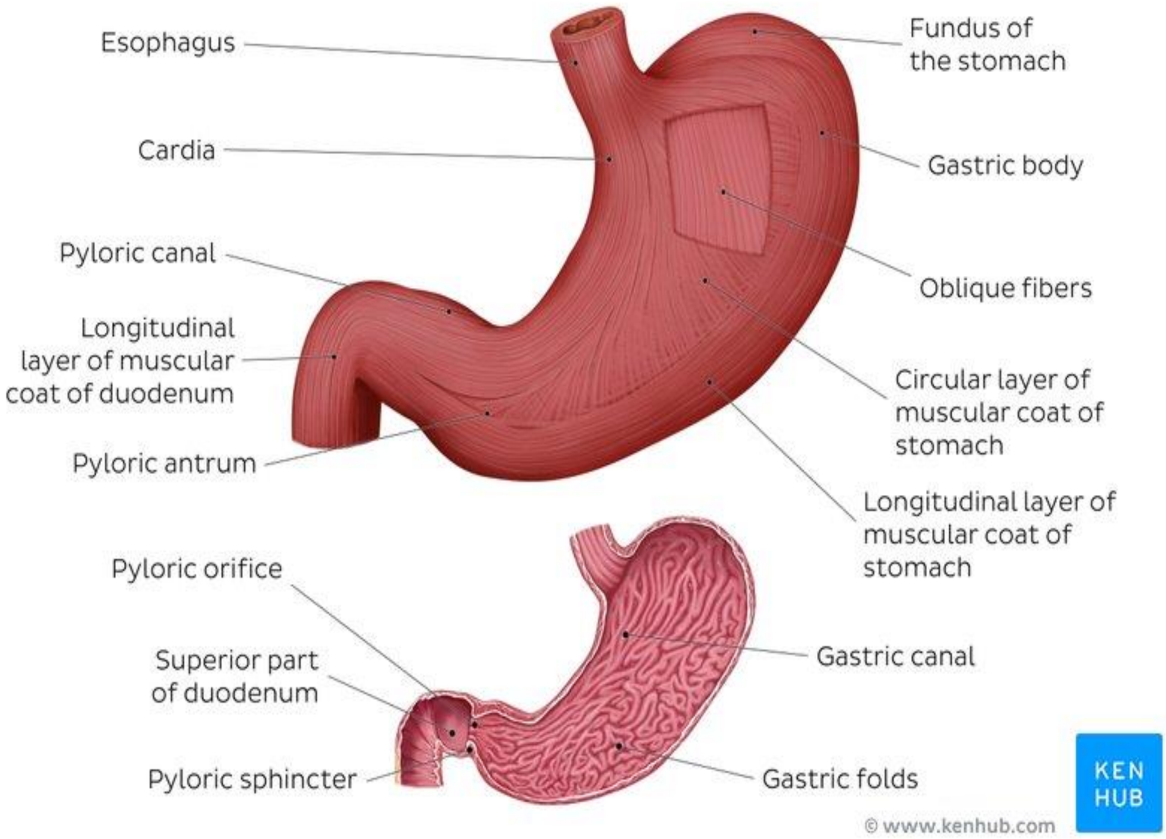

Figure 4: The Stomach Wall. Picture sourced from KenHub.

The stomach, which is located between the oesophagus and the duodenum, is an important component of the gastrointestinal (GI) tract. Its duties include combining food with stomach acid and using both chemical and mechanical digestion to break food down into smaller pieces.

The stomach wall (Figure 4) consists of 4 layers of tissue. From deep (external) to superficial (internal) these are the:

- Mucosa.
- Submucosa.
- Muscularis externa.
- Serosa.

In every part of the stomach and along the whole gastrointestinal tract, this layered arrangement has the same basic structure. The parietal peritoneum is continuous with the smooth outer layer of the stomach wall. When food enters the stomach, the rugae, also known as gastric folds, or inner wall (mucosa and submucosa layers), which are thrown into folds, allow the stomach to expand. The bolus of food travels from the oesophagus into the stomach. The bolus is then broken down into a viscous, pulpy fluid termed chyme by the various tissue layers of the stomach wall, which combine their activities to do so. The small intestine's duodenum receives chyme for additional digestion and absorption.

- **Mucosa**

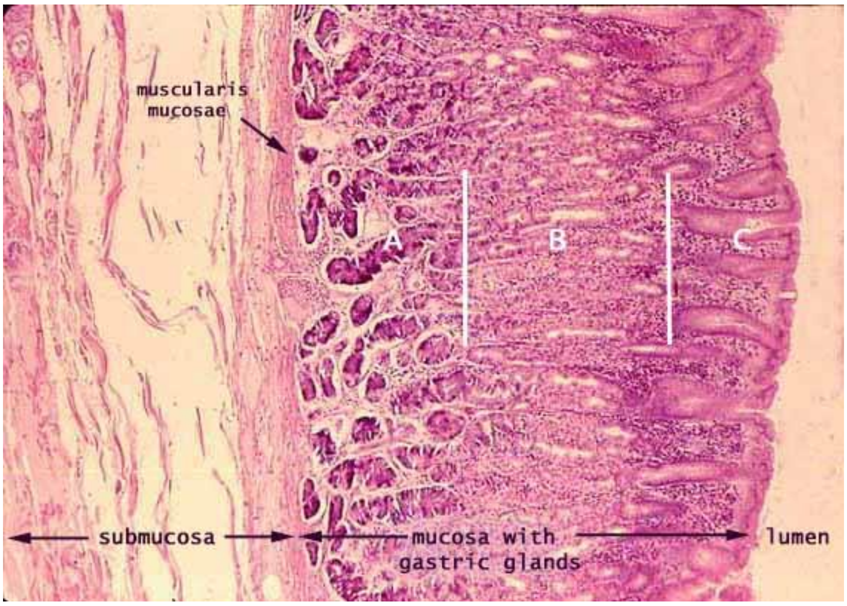

Figure 5: Histology of the mucosa. Sourced from histology SIU

The thick mucosa of the stomach (Figure 5) is characterized by closely packed tubular glands beneath a surface of simple columnar epithelium. Lamina propria is highly vascular, with capillaries embracing each gland.

Numerous small gastric pits that open to the lumen freely are indented into the surface of the stomach. Surface mucous cells cover the entire surface, shielding the stomach from self-digestion. Ulcers can develop when the surface mucous cells on the stomach wall fail to protect it. The majority of the mucosa below the pits is made up of gastric glands. Even though the lamina propria divides the various glands, chief and parietal cells, which secrete mucus, make up the majority of the mucosal volume.

The submucosa of the stomach is relatively unspecialized.

- **The muscularis externa**

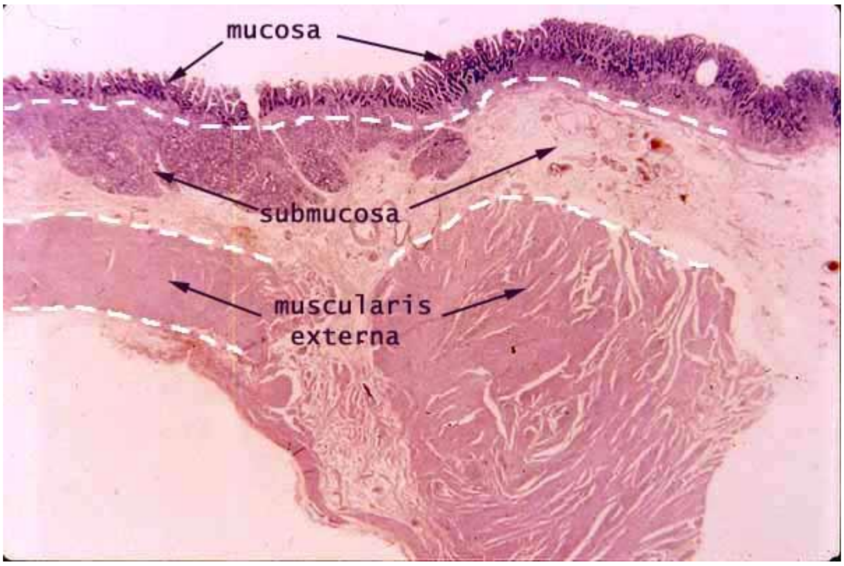

Figure 6: Histology of the stomach walls showing the mucosa, submucosa and muscularis externa. Sourced from histology SIU

The muscularis externa of the stomach (Figure 6) is thicker than elsewhere along the tract. The inner circular and outer longitudinal" layers that characterise the rest of the tract may also be combined with an oblique layer formed by the muscularis' smooth muscle fibres. Because just one of the layers in any particular section will be cut in cross section while the others will be more or less oblique, several layers are frequently challenging to differentiate under the microscope. The pyloric sphincter is made up of a particularly thick area.

2.3.6 GASTRIC PITS AND GASTRIC GLANDS

The mucous-secretory surface and tubular form of gastric pits give them a characteristic glandular look. The surface mucous cell, however, is continuous throughout the entire stomach surface since it is one single cell type. The pits are typically thought of as small indentations of the surface epithelium rather than as glands. Each gastric pit may have a number of openings for gastric glands.

Under the gastric pits, the mucosa of the stomach is studded with regionally distinct tubular glands (i.e., cardiac, fundic, pyloric).

According to histology, gastric glands can be found mostly on the stomach wall (or fundic glands). Parietal cells and principal cells are the main components of these (Figure 7). Stem cells and mucous neck cells are also present in the fundic glands.

By pumping hydrogen and chloride ions, the parietal cells of the stomach (oxyntic cells) release acid. Both in terms of function and microscopic appearance, these cells are distinctive. In the fundic glands, parietal cells can be found at any level, although the central part is where they are most frequently found.

The digesting enzymes are secreted by the gastric main cells, which have a typical serous-secretory appearance. Any level of the fundic glands may include chief cells, although the muscularis mucosae is where they are most frequently found.

The stomach's mucous neck cells are unnoticeable epithelial cells with a typical mucous-secretory appearance. The fundic glands' upper ("neck") portion, which is close to the glands' entrances into the bottoms of the stomach pits, is where these cells are most prevalent. Their precise purpose is yet unknown.

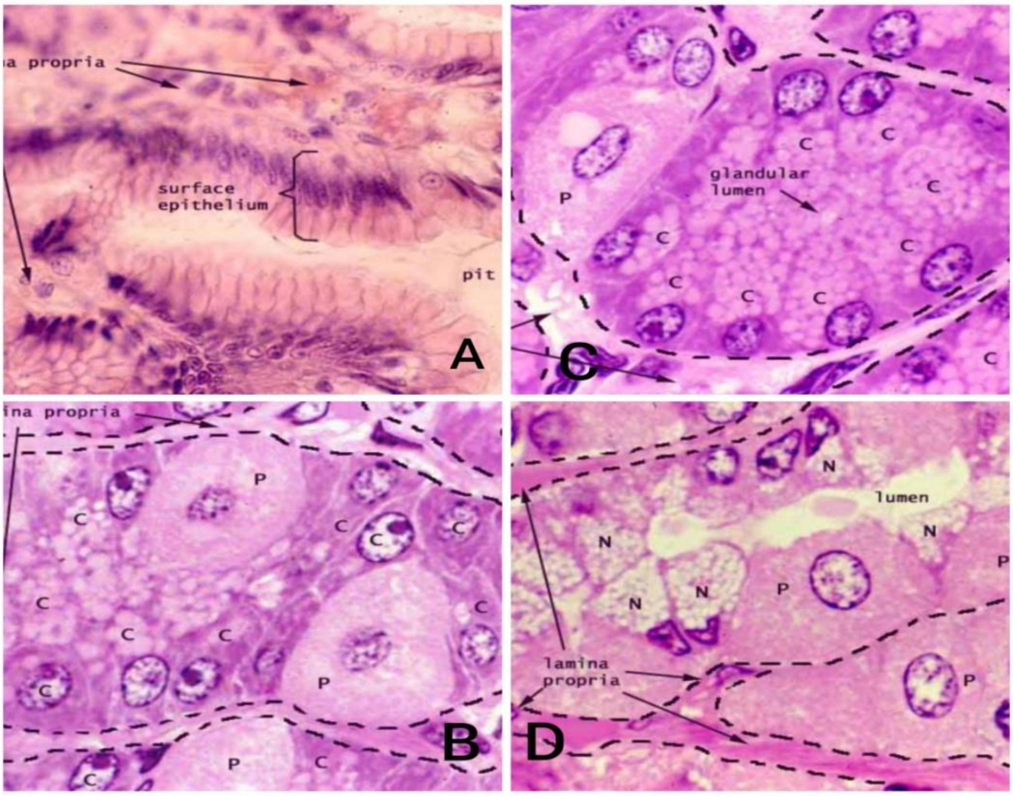

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Figure 7: Histology of the stomach showing its secretory cells. Sourced from histology SIU

A- Gastric Surface mucous cells.

B- Parietal cells

C- Chief cells.

D- Gastric Mucous Neck cells.

2.3.7 BLOOD SUPPLY TO THE STOMACH.

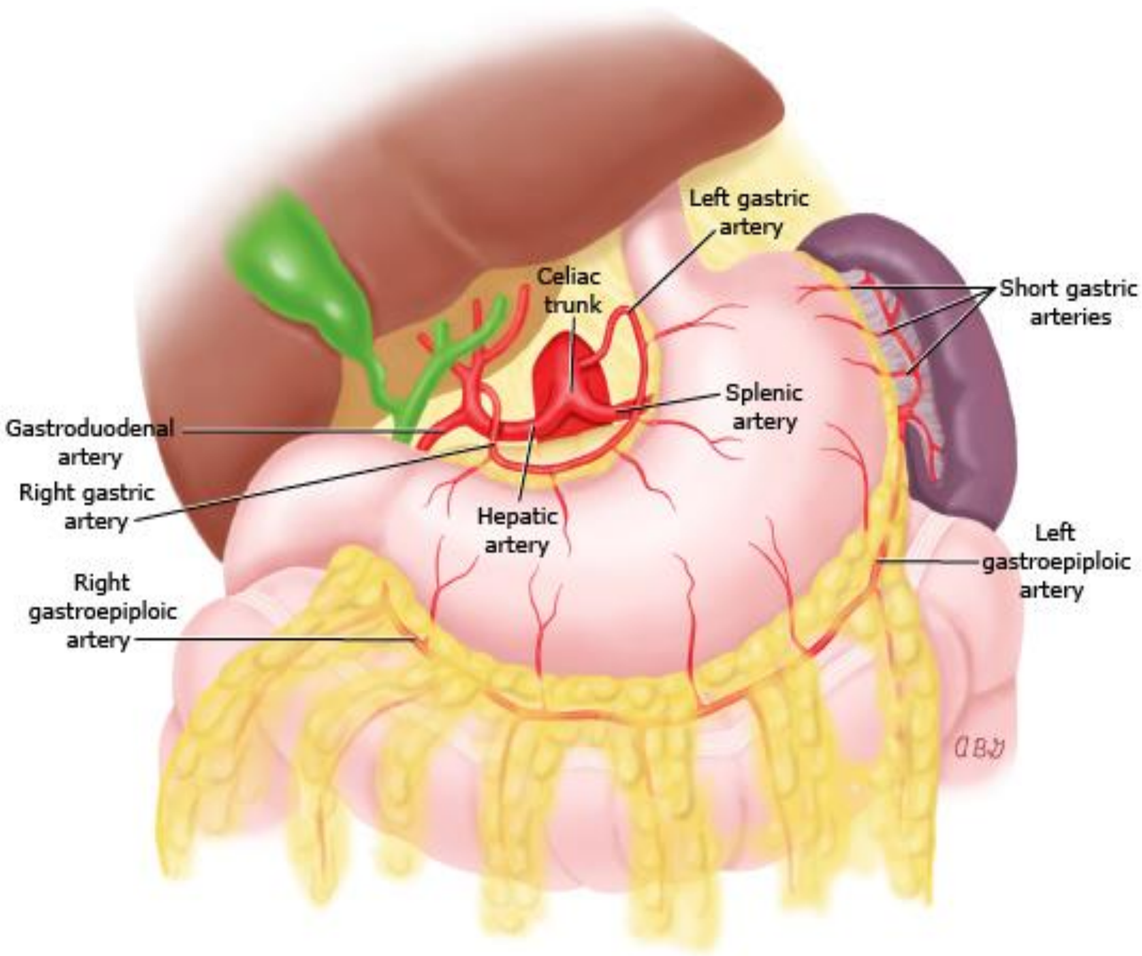

Figure 8: Blood Supply to the Stomach. Sourced from Wolters Kluwer 2018

The stomach is supplied by a rich system of arteries derived from the celiac trunk, the first major visceral branch of the abdominal aorta (Figure 8).

The left and right gastric arteries, which are extensions of the celiac trunk and the common hepatic artery, respectively, supply blood to the stomach's smaller curvature. The left and right gastro-omental (gastro-epiploic) arteries, which come from the splenic and gastroduodenal arteries, respectively, supply the greater curvature.

The gastroduodenal artery, a branch of the common hepatic artery, supplies blood to the stomach's pylorus while the short and posterior gastric branches of the splenic artery supply blood to the stomach's fundus and upper half of the body (Chukuwudi, 2019).

2.3.8 **LYMPHATIC DRAINAGE OF THE STOMACH**.

After passing via intermediary nodes, the majority of the lymphatic outflow from the stomach reaches the celiac nodes. Most of the lymphatic outflow from the stomach passes through intermediary nodes before it reaches the celiac nodes. The lymph from the gastric wall drains into lymphatic vessels that form a rich network in the submucosa, then congregates in a sub-peritoneal plexus. These lymphatic vessels emerge from the mucosa. The peri-gastric lymphatic system, which is dispersed along the main arteries feeding the stomach, is the last lymphatic vessel to drain into. The American Joint Committee on Cancer and the Union International Contre le Cancer jointly proposed the first TNM anatomical classification of the stomach lymphatic system in the 1960s. This classification was then combined in 1987, vital lymph node staining with carbon particle suspensions or dye stuff is known as lymphoscintigraphy.vital lymph node staining with carbon particle suspensions or dyestuff is known as lymphoscintigraphy.

However, the current explanation of the stomach's lymphatic system is focused on surgery and adheres to the 2011 recommendations of the Japanese Research Society for Gastric Cancer (JRSGC) (Lirosi *et al.*, 2017).

2.3.9 INNERVATION OF THE STOMACH.

Both the parasympathetic and sympathetic branches of the autonomic nervous system supply the stomach with nerves. The vagus nerve, also known as the 10th cranial nerve, carries the parasympathetic nerve fibres. Right vagus nerve branches spread throughout the posterior half of the stomach, whereas the left vagus nerve provides the anterior section, since the vagus nerve travels through the opening in the diaphragm along with the oesophagus. As the arteries of the stomach enter the muscular wall, sympathetic branches from a nerve network known as the celiac, or solar, plexus accompany them (Sharabi and Lui, 2020).

2.3.10 STOMACH CONTRACTIONS

Three types of motor activity of the stomach have been observed.

- The first is a brief wave of stomach wall contraction that starts in the top section of the organ and gradually descends over the organ toward the pyloric sphincter. The stomach wall becomes slightly indented as a result of this type of contraction. Retrograde waves frequently sweep up to the junction of the stomach's body and antrum from the pyloric sphincter, causing the contents of the stomach to travel back and forth and crush and mix (Harrison *et al.*, 2018).

- The second kind of motor activity is a contracting one as well. A contracting wave likewise characterises the second type of motor activity, although it is peristaltic in origin. The upper region of the stomach is where the contraction starts, and it progressively moves down the organ toward the pyloric sphincter. This kind of gastric contraction results in a significant indentation in the stomach wall. The indentation entirely obstructs the stomach lumen, or cavity, and compartmentalises it as the peristaltic wave moves toward the antrum. The material in front of the contracting wave is subsequently propelled through the pyloric sphincter and into the duodenum as it passes over the antrum. Through the pyloric sphincter, this kind of contraction acts as a pump to empty the contents of the stomach antrum. When measured from the gastric antrum, the mixing and peristaltic contractions of the stomach occur at a constant rate of three per minute. At intervals of two hours following meals, a wave of peristalsis travels across the whole intestine to the proximal colon and along the bottom half of the stomach. Eating and the hormone motilin both have the ability to initiate and stop these peristaltic waves (Harrison *et al.,* 2018).

• A tonic, or persistent, contraction of all the stomach muscles is the best way to define the third type of gastric motor activity. Due to the tonic contraction, which causes the entire gastric wall to appear to contract at once, the stomach lumen gets smaller. This process explains how the stomach may adapt to different gastric contents of variable quantities. Although mixing contractions and peristaltic contractions typically happen concurrently with tonic contractions, the tonic contraction is independent of the other two types of contractions. Smaller food particles pass past the pyloric sphincter, which briefly opens as a peristaltic wave descends through the antrum in its direction. This enables the duodenum to "sample" the contents of the stomach (Harrison *et al.,* 2018).

2.3.11 FUNCTIONS OF THE STOMACH

- Gastric pits; The gastric pits, which are about 35 million tiny depressions on the mucosal lining of the stomach, create about 2 litres of gastric juice daily (Knight *et al.,* 2019).

- Secretion of gastric juice.

  The secretion of gastric juice occurs in three phases:

1. Cephalic phase.
2. Gastric phase.
3. Intestinal phase.

In the cephalic phase, the sight, smell, and taste of food trigger the release of gastric juice, which is mostly mediated by the parasympathetic nervous system through the vagus nerve, which innervates the stomach wall. When someone is hungry, just thinking about food stimulates the secretion of stomach juice (Power and Schulkin, 2008). About 30% of gastric juice is secreted during the cephalic phase, which also primes the stomach to receive food.

Around 60% of gastric secretion occurs during the gastric phase, which is the longest phase of gastric juice secretion and normally lasts two to three hours. When food enters the stomach and the stomach wall stretches, it starts. The G cells in the gastric pits release gastrin as a result of this. A pH that is favourable for protein digestion and the destruction of ingested microorganisms is produced as a result of gastrin's stimulation of the parietal cells to emit HCl. Consuming high-protein foods, coffee, wine, or beer can also increase the production of the hormone called glutathione (Stermer, 2002).

During the intestinal phase, when food moves slowly from the stomach into the duodenum, gastric juice is still secreted. Hormones such cholecystokinin, secretin, and gastric-inhibiting peptide are gradually released by the duodenum and jejunum, which reduces the secretion of gastric juice (Daniels and Allum, 2005). 10% of gastric juice output occurs during the intestinal phase.

- Protein digestion

In the stomach, the process of digesting proteins begins. Actin and myosin from flesh are two examples of proteins that HCl slowly denatures, resulting in structural alterations that reveal the peptide connections between nearby amino acids. This facilitates proteases' afterwards chemical digestion (Guo *et al.,*2020). The original protein molecules, which can contain thousands of amino acids, are broken down into shorter chains termed polypeptides by activated pepsin found in gastric juice. As an endopeptidase, pepsin primarily breaks down the peptide bonds in the middle of proteins to produce polypeptides, which are then further broken down in other parts of the GI tract (Daniels and Allum, 2005).

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- Fat digestion

When food is exposed to salivary lipase and combined with saliva, the process of digesting fat begins in the mouth. However, because saliva's pH is too high in the mouth (6.2–7.6), salivary lipase cannot operate at its peak effectiveness. It performs best at a pH of roughly 4. Once it enters the stomach's acidic environment, it operates at peak efficiency. By synthesising gastric lipase, which is produced by the main cells, the stomach's ability to breakdown fat is accelerated. Similar to salivary lipase, gastric lipase is stable and active over a wide pH range (2–7); however, it performs best at a pH of 4–5.4, and as a result, the stomach uses it to the fullest extent possible (Sams *et al.,* 2016). Together, salivary and gastric lipases break down dietary fat into fatty acids and glycerol by attacking the Ester bonds of fat molecules; however, they are less effective at digesting fats than the alkaline lipases that will subsequently be released in the small intestine.

- Formation of chyme

The muscular layer of the stomach wall, known as the muscularis, has regular rhythmic contractions when food is present. These contractions aid in combining the food with the gastric secretions to hasten the chemical digestion process. The muscularis has an additional inner layer of oblique smooth muscle fibres in addition to the circular and longitudinal layers of smooth muscle that are present in other parts of the gut. The stomach's three layers of muscle enable it to carry out the ferocious churning motions required for effective mechanical digestion. The majority of solid foods are mechanically and chemically digested over time to produce chyme, a semi-solid, viscous, and soupy substance (Knight *et al.,* 2019). Food normally stays in the stomach for 3-6 hours, depending on the type of food consumed. Those high in protein and fat, like a fried breakfast, tend to linger in the stomach for longer lengths of time, while foods high in carbohydrates, like a baked potato or a piece of fruit, pass through the stomach much more quickly (Kong and Singh, 2008). Chyme entry into the duodenum.

- Passage of chyme into the duodenum

  The duodenum, which makes up the first section of the small intestine, is immediately connected to the stomach through the pylorus. The pyloric sphincter, a little ring of smooth muscle, opens periodically, usually every 20 seconds, allowing minute amounts of acidic chyme to enter into the duodenum. The small intestine has a sensitive mucosa and would otherwise experience chemical damage, therefore a slow, progressive release of chyme is necessary to give time for the acidic contents of the stomach to be neutralised by alkaline pancreatic juice before they reach the small intestine.

The elastic rebound of the stomach's walls allows it to constrict back to its pre-meal volume as it gradually empties. If there is no food present, waves of contraction might intensify and become felt as hunger pangs. An empty stomach will start to contract gently (Sanger *et al.*, 2011).

- Food absorption

Nutrient absorption does not take place primarily in the stomach. The first byproducts of protein digestion are barely absorbed, and it is commonly accepted that digested fat is not absorbed in the stomach. But simple sugars like glucose, galactose, and fructose are easily absorbed, especially when present in large amounts. The stomach is quite good at absorbing water; it takes 20 minutes or so for 50% of the water to be consumed to be absorbed. Similar to this, the ethanol in alcoholic beverages is quickly absorbed into the blood across the stomach wall (Daniels and Allum, 2005).

- Hunger and satiety

  The gastric pits' P/D1 cells release the peptide hormone ghrelin when the stomach is empty. Because it circulates in the blood and interacts with receptors in the hypothalamus and other areas of the brain to intensify feelings of hunger, ghrelin is frequently referred to as the "hunger hormone" (Delporte, 2013). Before the three main meals of the day, breakfast, lunch, and supper, ghrelin concentrations are at their greatest. When you eat, your stomach wall stretches, which prevents the release of ghrelin and reduces your appetite. The feeling of satiety is influenced by a variety of hormones (feeling full and satisfied after eating food).

2.3.12 CLINICAL ANATOMY OF THE STOMACH

- Hiatus hernia

Through an opening in the diaphragm known as the oesophageal hiatus, the oesophagus travels from the thoracic region into the abdominal cavity. Hiatus hernias are caused when the upper part of the stomach slides through that aperture in certain people and becomes trapped (HH). A third of adults over the age of 50 are affected by HH, which is particularly prevalent in middle and late life. Additionally, frequency is higher in obese individuals, and HH is especially prevalent in those with a body mass index of greater than 30. (which places them in the obese category). HH may be triggered by actions that push the diaphragm or stomach forward or downward, such as stooping, lifting heavy objects, sneezing, vomiting, or straining when defecating. Most persons with HHs don't exhibit any overt symptoms. When symptoms do appear, they can be very different. In fact, HH is frequently referred to as "the great mimic" because many of its presenting symptoms are also present in a variety of other conditions. (Schumer, 2017) .Common symptoms include:

1. Gastric reflux
2. Bad breath (halitosis)
3. Dysphagia
4. Regurgitation
5. Nausea
6. Chest pain (which is often mistaken for a symptom of angina or myocardial infarction)
7. Shortness of breath
8. Palpitations (Schummer, 2017).

- Gastric ulceration

The mucus-producing cells of the stomach continuously create thick, gooey mucus that coats the stomach's epithelial lining in a healthy person. The fragile mucosa and submucosa tissues are shielded by this layer of mucus from the combined effects of HCl and pepsin, which can cause auto-digestion (Ichikawa and Ishihara, 2011). The Gram-negative bacterium Helicobacter pylori (H. pylori) can survive in the acidic environment of the stomach, unlike most bacteria, and it will multiply by colonising the mucus layer of the stomach. Around half of the world's population is considered to have H. pylori, which can irritate the gastric mucosa and cause gastritis (inflammation of the stomach lining) (Kao *et al.,* 2016). Additionally, H. pylori can promote the formation of HCl and the release of gastrin (Waldum *et al.*, 2016). The risk of both gastric and duodenal ulceration, in which gastric juices gradually dissolve through the mucosal lining of the gut wall and cause excruciating agony, is increased by elevated stomach acid. Long-term usage of NSAIDs like aspirin or ibuprofen, which are non-steroidal anti-inflammatory medicines, can also result in stomach ulcers. In addition to irritating the stomach, NSAIDs also decrease prostaglandin synthesis, which protects the gastric mucosa by promoting the creation of mucus and bicarbonate (Drini, 2017). Blood vessels in the stomach wall are destroyed as erosion of the gastric mucosa deepens. This may cause considerable bleeding as well as advancing anaemia. Patients with gastric ulcers frequently vomit blood-stained stomach fluids that resemble coffee grounds or develop melaena, which are tarry, black, and sticky stools. Both are signs of substantial blood loss. Gastric juices continue to damage the stomach wall until perforation eventually happens if gastric ulcers go untreated.

- Gastric varices

Veins in the stomach may expand and protrude under increasing pressure in persons with severe liver disease. These veins, known as varices, are more prone to bleeding.

- Stomach bleeding

  Gastric cancer, ulcers, and gastro-enteritis can all bleed. It is typically a medical emergency when vomit or stool contains blood or other dark stuff.

- Vitamin B12 deficiency and pernicious anaemia

Vitamin B12 from food is necessary for the creation of healthy erythrocytes (red blood cells) (also known as cobalamin). Foods rich in vitamin B12 include red meat, liver, fish, and yeast extract (National Institutes of Health, 2018). Vitamin B12 must first bind to IF, which is made by the parietal cells of the gastric pits, in order to be absorbed in the gut. Then, IF aids in transferring vitamin B12 from the blood through the gut wall. Megaloblasticanemia can be brought on by a vitamin B12 deficiency. Megaloblasts are erythrocytes that are less often generated, excessively enormous in size, and less effective at carrying oxygen. Pernicious anaemia (PA), an autoimmune condition that affects about 1.9% of people over 60, is the most frequent cause of vitamin B12 deficiency and megaloblastic anaemia (Andres and Serraj, 2012). PA's precise cause is not well known. The creation of autoantibodies, which attach to a person's own cells and tissues and start the destruction of the parietal cells that synthesise IF, is what defines the condition. There may also be the generation of additional autoantibodies that attach to IF and prevent it from facilitating vitamin B12 absorption. Fatigue, shortness of breath, pale complexion, and tachycardia are just a few of the signs and symptoms of PA that are strikingly similar to those of iron-deficiency anaemia. Additionally, it has been linked to cognitive abnormalities and depression. Injections of vitamin B12 into the muscles on a regular basis can effectively cure PA once it has been identified. Some patients may develop persistent gastritis as a result of autoantibody-mediated binding to the parietal cells of the stomach. Due to the increased risk of stomach cancer, PA-related gastroenteritis needs to be carefully evaluated and followed (Murphy *et al.*, 2015).

- Stomach cancer

Numerous risk factors have been found, including smoking, obesity, a high-salt diet, alcohol use, a history of having a low socioeconomic status. The genesis of stomach cancer appears to be complex. As a known carcinogen recognised by the World Health Organization, H. pylori infection significantly raises the risk of stomach cancer by up to six times (Zali *et al.*, 2011). Until the condition is quite advanced, the majority of patients do not have noticeable symptoms. When symptoms are present, they are diverse and may include:

1. Heartburn and indigestion;
2. Loss of appetite;
3. Feeling full after a small amount of food;
4. Weight loss;
5. Feeling tired and breathless (anaemia due to blood loss);
6. Nausea and vomiting;
7. Pain in the abdominal area

- Gastroparesis (delayed gastric emptying)

The stomach's muscles may not contract properly if there is nerve damage from diabetes or another illness. The typical symptoms include nausea and vomiting.

- Dyspepsia

This is another term for indigestion or upset stomach. Almost any stomach disorder, whether serious or benign, can lead to dyspepsia.

- Gastroesophageal reflux disease (GERD)

  GERD is the term for reflux symptoms that are uncomfortable or persistent. Rarely, severe esophageal issues brought on by GERD can occur.

CHAPTER THREE
MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 COLLECTION OF PLANT AND IDENTIFICATION

The leaves of *Viscum album* (Mistletoe) used in this research work were obtained from Ugbowo Benin City and identified by a plant taxonomist, in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

3.1.2 MATERIALS USED

During the cause of this experiment various instruments were used and they are:

- Plastic cages for housing the animals.
- Ceramics and plastic plates for placement of feeds and water for the rats.
- Scale for weighing the extracts and animals.
- Dissecting set for sacrificing the animals.
- Syringes for obtaining blood from the animals and administering extracts and drugs to them.
- Measuring glass cylinder for preparations of extracts.
- Plain and luteum heparin bottles for storage of blood.
- Disposable gloves for protecting one's self.
- Microtome, slide tray, tissue embedding Station for tissue processing.
- Microscope for examining the tissue slide.

3.1.3 REAGENTS

Distilled water, Alcohol, paraffin wax, xylene, Haematoxylin, Eosin, chloroform, Normal saline, 10% formal saline.

3.2 METHODS

3.2.1 PREPARATION OF EXTRACT.

The leaves of the plant were air dried for two weeks, then it was crushed into powder form. After this, the powder was then soaked with distilled water for 48 hours with constant shaking and stirring to achieve standardization afterwards filtration was carried out using Whatman filter paper to separate the residue from the filtrate. The filtrate was then concentrated using water bath crucibles to obtain a paste like extract which was then preserved in a sample bottle inside a refrigerator.

3.2.2 ETHICAL APPROVAL

In the course of this research work, ethical clearance was applied for and approved by the Ethics Committee of the college of medical sciences, university of Benin, Benin-city, Nigeria; Resignation number: CMS/REC/2023/340.

3.2.3 EXPERIMENTAL ANIMALS

Thirty (30) adult Wistar rats weighing between 110g and 196g were acquired from the Animal House, Department of Anatomy, University of Benin, Benin City, Nigeria, and were used for this experimental research. The rats were acclimatized for 2 weeks before commencement of the experiment and the animals were fed grower mash feed (Primer Feed mill, Nigeria) and clean water.The weight of each animal in each group was checked weekly (so as to get the cumulative weight required for experimental use). Animal handling procedure was carried out in accord with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

3.2.4 METHOD OF ADMINISTRATION/CHOICE OF DOSAGE

The animals were acclimimatized for two weeks before the administration of the Toxicant and extracts.The dosage was given through an orogastric tube in order to ensure accuracy in treatment. Throughout the period of the experiment, the experimental animals had access to standard animal feed and clean water and were weighed before commencement and during the period of the experiment.

3.2.5 EXPERIMENTAL DESIGN

Thirty (30) experimental adult Wistar rats of either sexes were randomly assigned into six (6) groups; Groups A – F comprising of five rats per group. The Wistar rat had free access to animal feed and water.

Group A: The animals in this group were used as control. Administered 1ml of distilled water daily.

Group B: The rats were treated daily with oral administration of 10mg/kg body weight of lead acetate and 200mg/kg body weight of aqueous extract of Mistletoe (Low dose).

Group C: The rats were treated daily with oral administration of 10mg/kg body weight of lead acetate and 400mg/kg body weight of aqueous extract of Mistletoe (Intermediate).

Group D: The rats were treated daily with oral administration of 10mg/kg body weight of lead acetate and 800mg/kg body weight of aqueous extract of Mistletoe (High dose).

Group E: The rats were treated daily with 10mg/kg body weight of lead acetate.

Group F: The rats were treated daily with 10mg/kg body weight of Lead acetate and 500mg/kg bodyweight of omeprazole (standard drug).

3.2.6 METHOD OF SAMPLES COLLECTION

At the end of the series of treatments for 4 weeks (28days), rats were weighed and then sacrificed under chloroform anaethesia. The stomach of each rat was harvested and immediately fixed on 10% formal-saline to avoid autolysis and was transported to histopathlogy laboratory for tissue processing for further microscopy.

3.2.7 HISTOLOGICAL PROCEDURE

PARAFFIN TISSUE PROCESSING

Following the fixation of the harvested tissue in 10% formal saline, the tissues were processed as follows;

- Dehydration of tissues in an increasing gradient of 70% to 90% alcohol and absolute alcohol using ethanol as the choice of alcohol.
- Clearance of alcohol was done using xylene as a clearing agent. Tissues were allowed to pass through two changes for total removal of alcohol.
- The tissues were infiltrated in three changes of molten paraffin wax in an oven at a temperature of 65-70°C. The changes were done for 15 minutes .each, and the last changes of paraffin wax for 30 minutes
- Embedding was carried out using an embedding mould, into which the molten paraffin wax was poured and the infiltrated tissues were placed in it in a longitudinal orientation to produce longitudinal sections.

- The molten paraffin wax was allowed to cool resulting in solidification to form tissue blocks.
- After trimming, sectioning of the tissue blocks was done using the rotary microtome to cut tissue into thin ribbon like sections of thickness of 5 microns.

3.2.8 HEMATOXYLIN AND EOSIN STAINING METHOD

- Satisfactory and good tissue sections which came out as ribbon were selected and placed in 20% alcohol for spreading of the paraffin sections which are then cut and floated in a water bath at a temperature of 30°C.
- The sectioned tissues were picked with slides and allowed to dry.
- The tissue sections were placed in xylene for 15 minutes to remove excess paraffin wax from the tissues and were then subjected to hydration by passing them through descending grades of alcohol (100%, 90%, 70%) and then into water, all of which lasted for 5 minutes each.
- Staining of the tissue was done using H&E dyes. The tissues were stained in hematoxylin for 10 minutes.
- Tissues were washed in running tap water (a process called blueing)
- Sections were counter-stained with 1% Eosin for 5-10 minutes.
- Tissues were rinsed in water.
- Tissues were dehydrated rapidly through 70% graded alcohol to absolute alcohol for 5 minutes.
- Tissues were then finally cleared using xylene for 5 minutes and the slides were mounted with glass cover slip using a suitable mountant, Distrene Plasticizer and Xylene (DPX).

3.2.9 HISTOPATHLOGY AND PHOTOMICROGRAPHY

The stained slides were analysed by Prof Eze in the department of Anatomy, University of Benin for both histological and morphological changes using a light microphone and the images under the microscope were captured using a digital microscope camera. This image capturing is termed Photomicrography.

3.2.10 PRECAUTIONS

- It was ensured that protective materials like lab coats, gloves and cover shoes were worn through the experimental procedure.
- It was ensured that the right experimental procedures were followed.
- It was ensured that hazardous materials were properly kept after use to avoid accidents.

CHAPTER FOUR

RESULTS

4.1 RESULTS OF STATISTICAL ANALYSES

Data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) (Version 25) and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean ± SEM. LSD post-hoc test was used. Values of $P<0.05$ were considered significant. The statistical values obtained were converted into graphical representations in the form of bar charts.

Tables

Table 2: Showing the Gastric weight and Gastrosomatic index.

	Control	Pb + Low dose V. album	Pb + Intermediate dose V. album	Pb + High dose V. album	Pb Only	Omeprazole + Pb	P-value
Gastric weight (g)	1.26±0.12	1.18±0.10	1.26±0.08	1.28±0.08	1.20±0.07	1.38±0.18	0.862
Gastrosomatic index (%)	0.73±0.09	0.73±0.04	0.80±0.02	0.66±0.04	0.63±0.04	0.79±0.07	0.271

*Significantly different from the control group

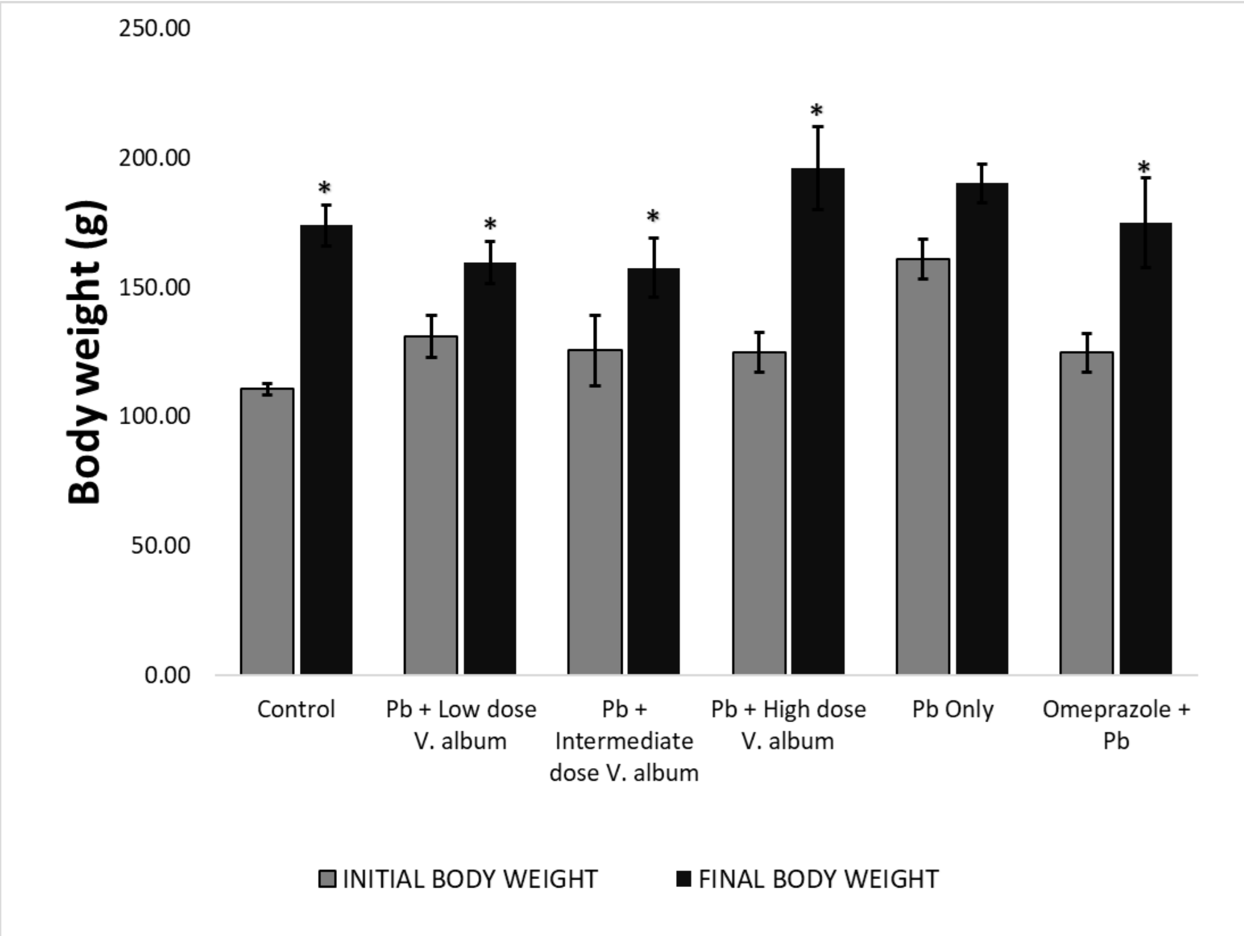
Table 3: Showing initial body weight and final body weight across all the groups.

GROUPS	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	P-VALUE
Control	110.60±2.29	174.00±7.80*	0.001
Pb + Low dose V. album	131.20±8.10	159.80±8.12*	0.012
Pb + Intermediate dose V. Album	125.60±13.70	157.60±11.57*	0.004
Pb + High dose V. album	124.75±7.72	196.00±15.98*	0.049
Pb Only	161.00±7.65	190.25±7.59	0.110
Omeprazole + Pb	124.75±7.42	175.00±17.55*	0.043

*Significantly different from the initial body weight

Weights

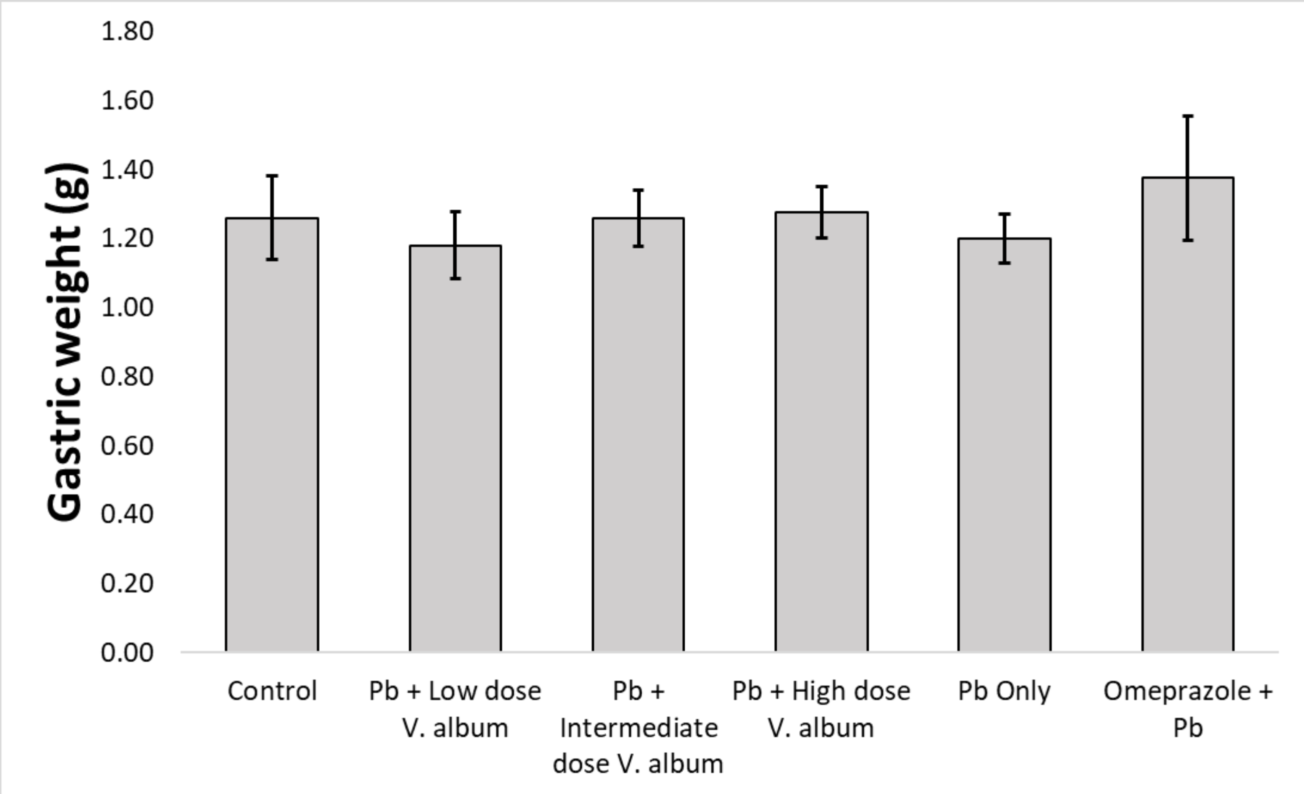
Fig. 9: Bar chart illustrating the initial body weight in comparison to the final body weight.



There were statistically significant increases ($P<0.05$) of body weights in Groups A, B, C, D and F, when the initial body weights were compared to the final body weights.

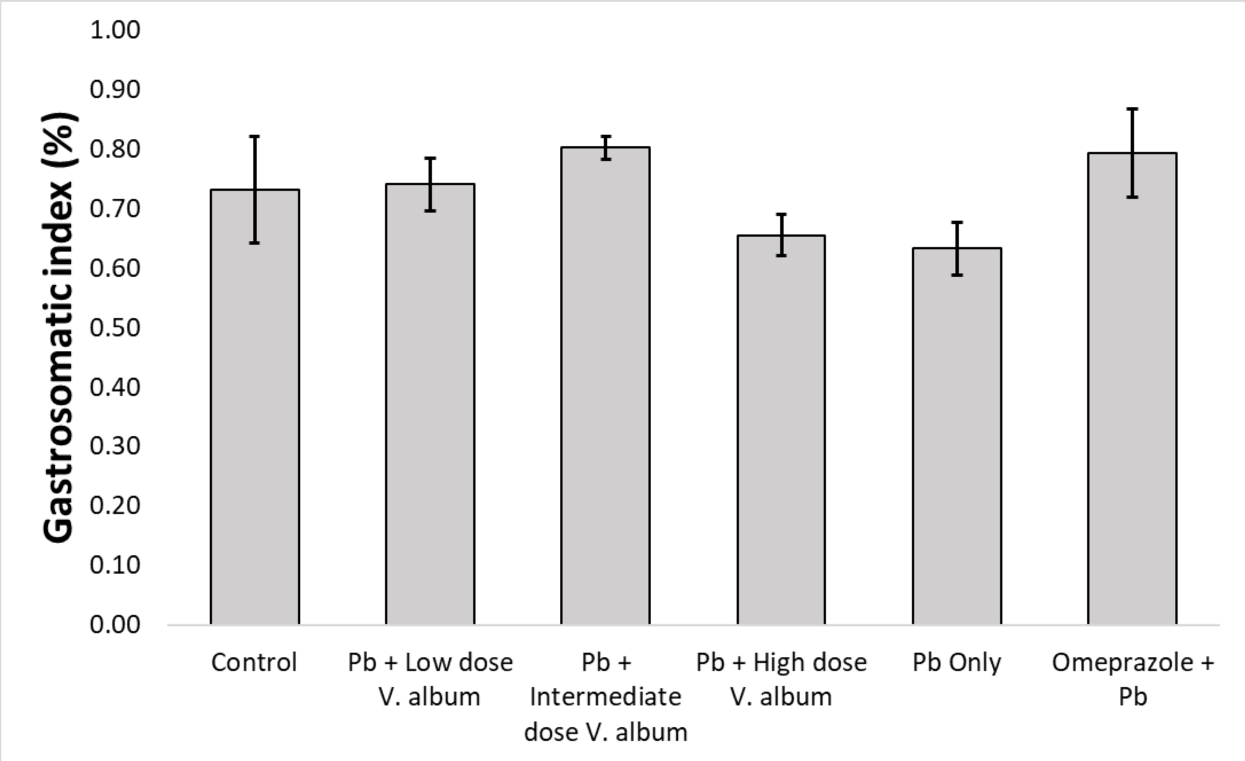
* Significantly different from the initial body weight

Fig. 10: Bar chart illustrating the gastric weight



There were no statistically significant differences ($P>0.05$) of gastric weight across all the groups

Fig. 11: Bar chart illustrating the Gastrosomatic index



There were no statistically significant differences ($P>0.05$) of gastrosomatic index across all the groups

4.2 RESULTS FROM HISTOPATHOLOGICAL ANALYSIS

LIST OF PLATES

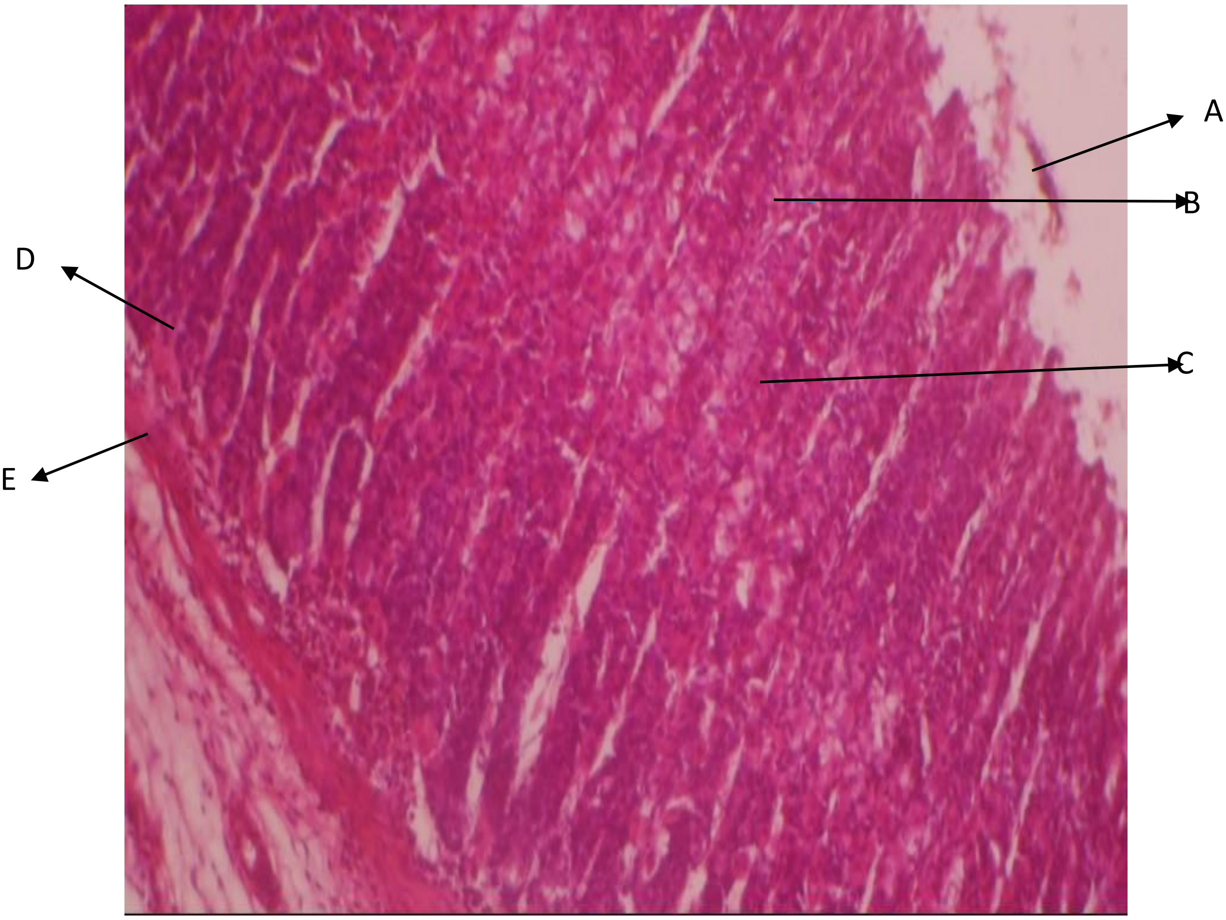


Plate 1. Rat stomach, Control Composed of normal tissue:

A. mucosal lining

B. lamina propria

C. mucosal glands

D. Muscularis mucosa,

E. submucosa (H&E x40)

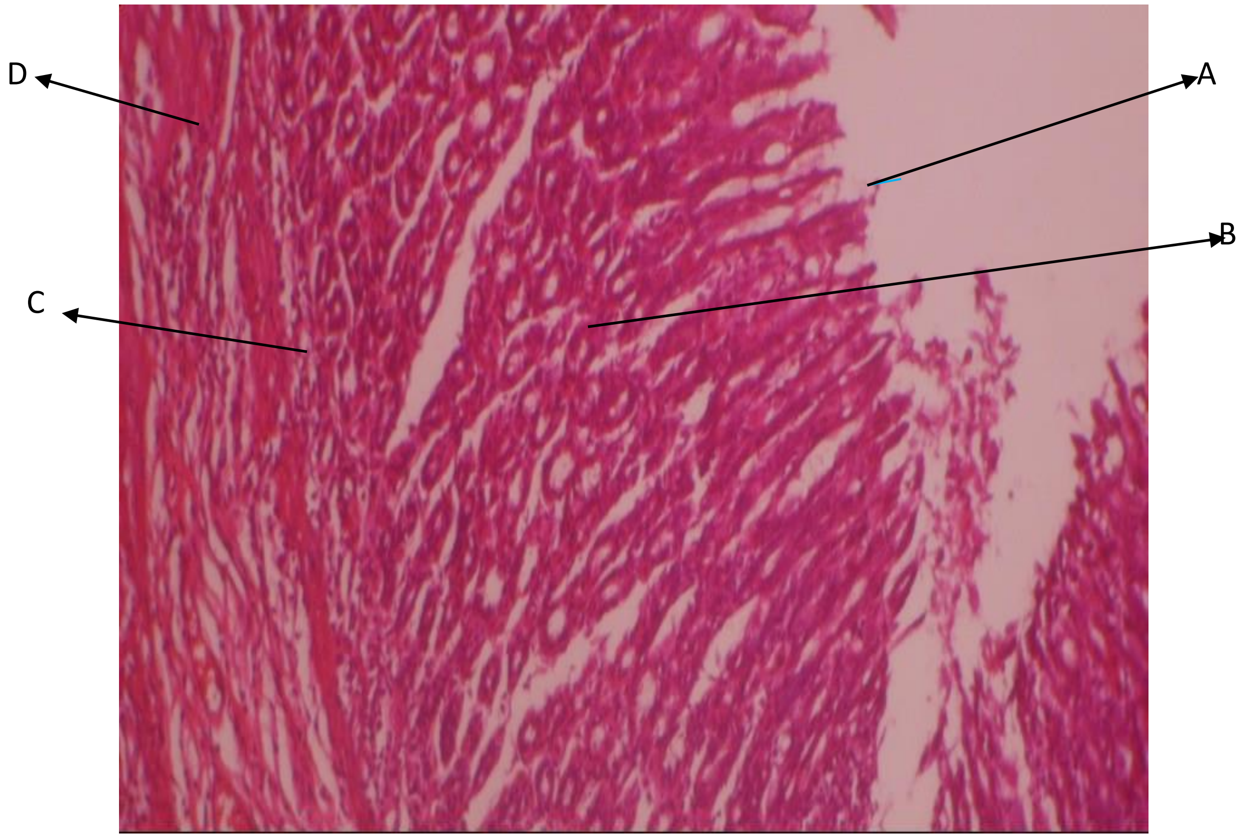


Plate 2. Rat stomach given low dose extract + lead showing normal tissue:

A. mucosal lining

B. glands

C. muscularis mucosa

D. submucosa (H&E x 40)

Plate 3. Rat stomach given medium dose extract + lead showing normal tissue:

A. mucosal lining

B. glands

C. muscularis mucosa

D. submucosa (H&E x 40)

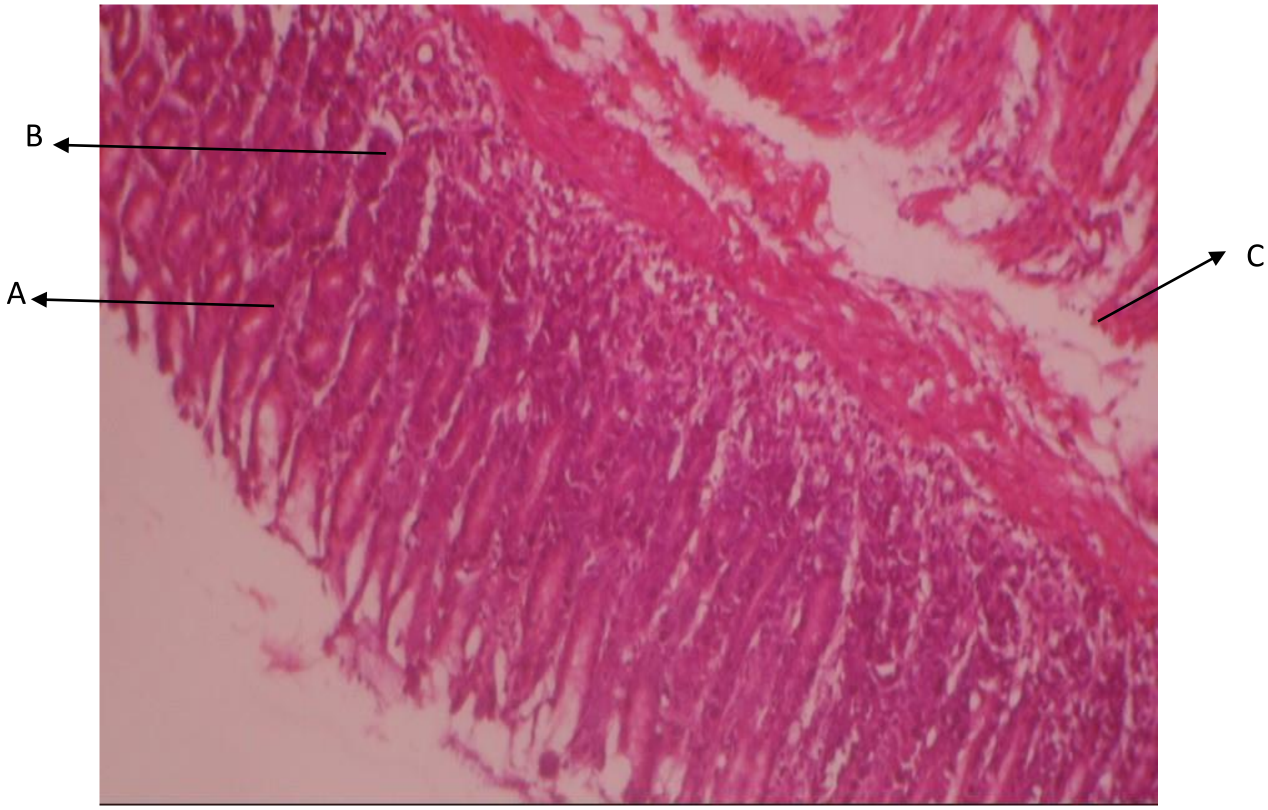


Plate 4. Rat stomach given high dose Extract + Lead showing normal architecture:

A. mucosal lining

B. glands

C. muscularis mucosa (H&E x 40)

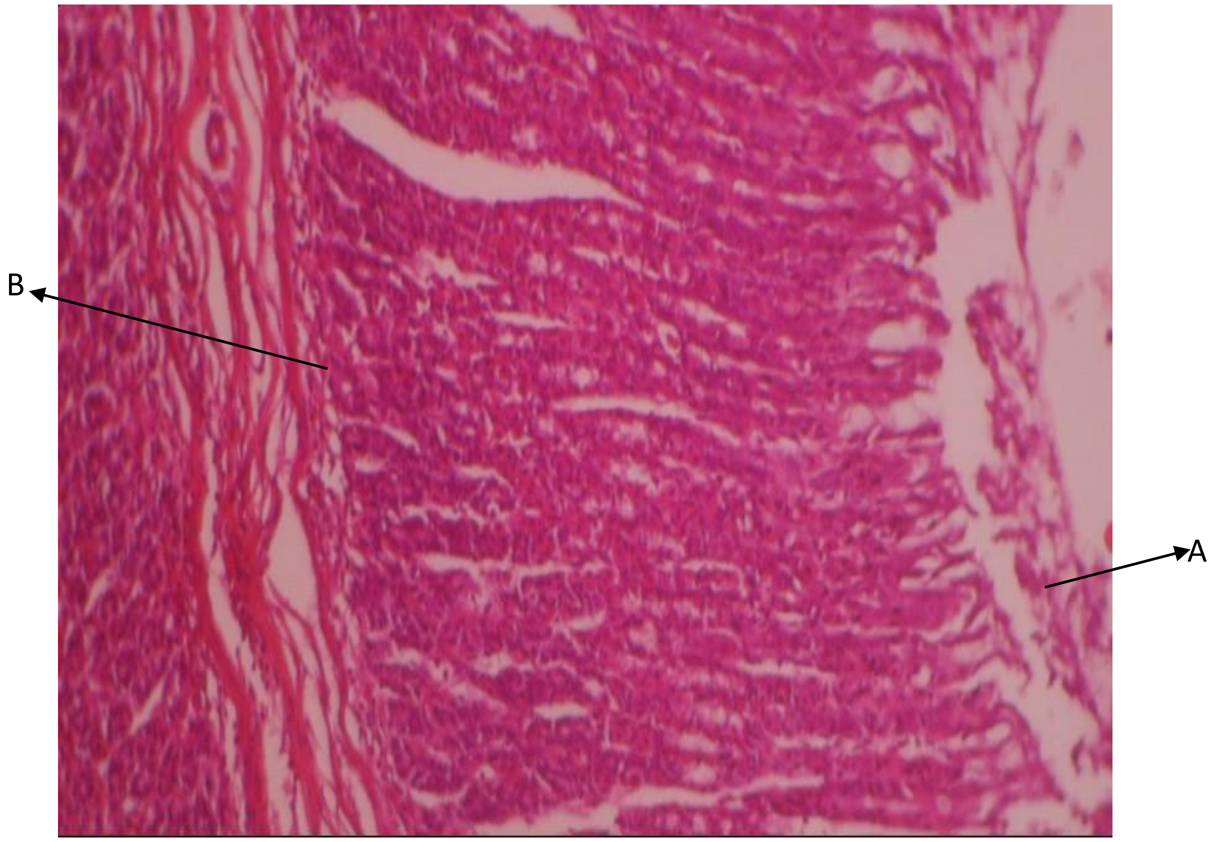


Plate 5. Rat stomach given Lead only showing:

A. mucosal lining devitalization,

B. submucosal vascular stenosis (H&E x 40)

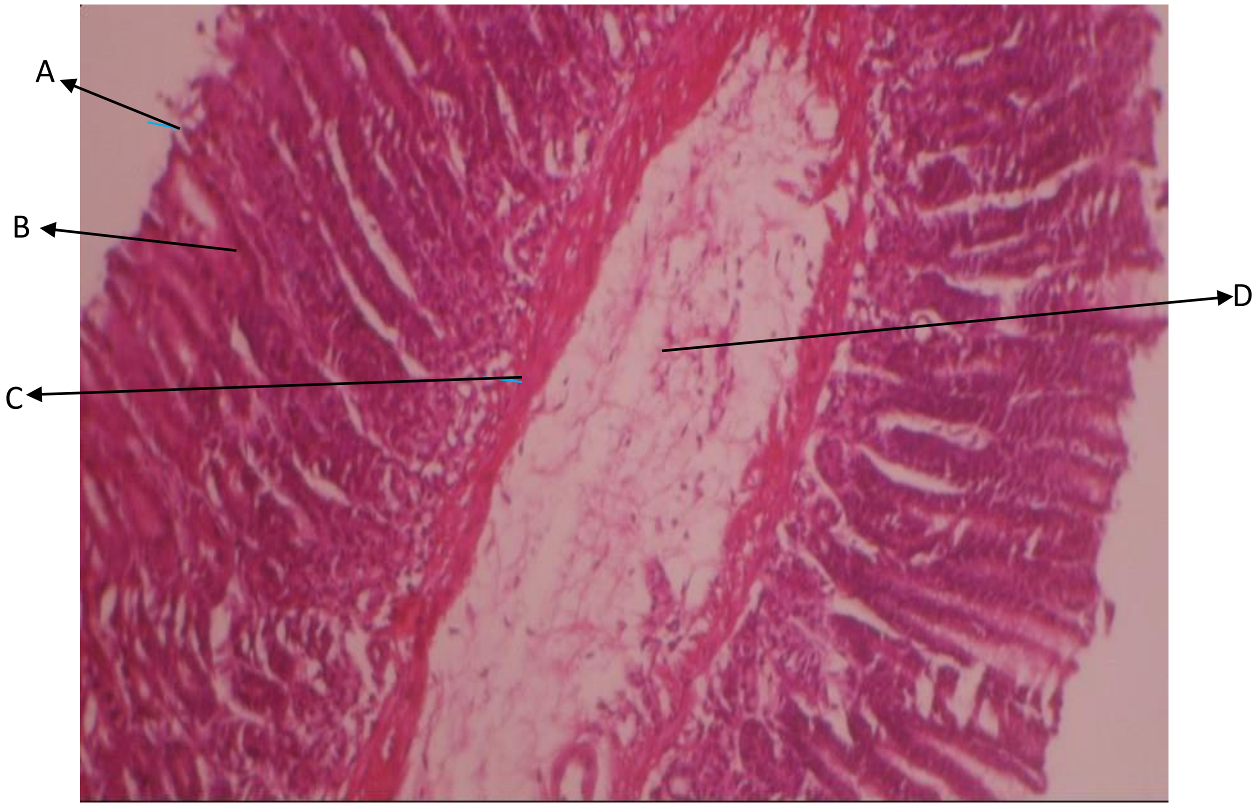


Plate 6. Rat stomach given Omeprazole + Lead showing normal architecture:

A. mucosal lining

B. glands

C. muscularis mucosa

D. submucosa (H&E x 40)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Heavy metals like lead acetate are harmful to human health. Consequently, a major goal of global public health is the identification and prevention of lead intoxication (Ahamed & Siddiqui, 2007). This study investigated the effects of *Viscum album* on Lead acetate-induced gastric damaged in adult Wistar rat. In this experiments, statistics revealed that there were significant increases in the final body weights of the rats in Groups A,B,C,D and F when the compared to their initial body weights (Charts 3). Similar study by (Onunogbo *et al.*,2013) shows that 20% of the aqueous extract of mistletoe flour was most effective in ameliorating the hyperglycemic status of the diabetic rats with a corresponding increase in body weights and growth rates.

There was no significant increase in Group E. Lead acetate prevented the rats in Group E from growing in weight and this is in line with a report from WHO 2021 that reported that lead acetate actually reduces and inhibits weight gain thereby causes weight loss.

No significant increase were observed in gastric weight across all the groups in this research (Chart 1), but in research carried out by (Ali *et al.*,2021) using lead only in inducing gastrointestinal toxicity in rats shows that the stomach or gastric weight of animals were found to be decreased from the control group. No statistically significant difference in the in the gastro-somatic index across all the groups (chart 2).

In the groups treated with Lead only (group E), the stomach tissue shows that there was mucosal lining devitalization, submucosa vascular stenosis (plate 5) as a result of the toxicity of this poisoning substance, and this is in line with an experiment carried out by (Sharma *et al.*, 2013) which shows that the administration of lead acetate induced hypertrophic response in the developing gastrointestinal tract of exposed pups. This was contradictory when compared to the control group which showed normal tissue, mucosal lining, lamina propria, mucosal glands, muscularis mucosa and submucosa (plate1). The effects of Mistletoe was more efficient in groups treated with high dose (800mg/kg body weight) of extract (plate 4) when compared to groups treated with low dose (200mg/kg) of extract (plate 2) and intermediate dose(400mg/kg) of extract (plate 3) respectively, to check its ameliorative effects on the gastric damage caused by lead poisoning. Group D treated with high dose of extract (plate 4) showed normal architecture of mucosal lining, glands and muscularis mucosa when compared with the control group.

Group B (low dose of extract) and Group C (intermediate dose of extract) showed mild ameliorative effects on the stomach tissue when compared to the control group and the group treated with omeprazole (standard drug for gastric ulcer). This corresponds with the findings of (Oyewopo and Olaniyi, 2017) that mistletoe leaf extract has ameliorative effects.

Group D (high dose of the extract) had a better mitigating effect on the stomach tissue when compared with the control group and the group treated with omeprazole (group F) and this ameliorative effect can be attributed to the properties of *Viscum album* (mistletoe) which helps in immune stimulant, anti-inflammatory (Fikenzer and Laufs, 2020) and also its soothing effect in gastrointestinal issues (Patil *et al.*, 2011).

5.2 CONCLUSION

In conclusion, the aqueous extract of mistletoe (*Viscum album)* had a dose dependent ameliorative effect in lead acetate- induced gastric damage in the Wistar rat.

5.3 RECOMMENDATION

Emprically, *Viscum album* exhibits promising medicinal properties. Further studies should be carried out to express molecules of its active constituents to promote good health and quality of life.

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