

**INVESTIGATING THE EFFECTS OF AQUEOUS LEAF EXTRACT OF *Camellia sinensis* (GREEN TEA) ON ARSENIC TRIOXIDE INDUCED GASTRIC DAMAGE  
IN ADULT WISTAR RATS**

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**NOVEMBER, 2025.**

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**BEING A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ANATOMY,  
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**NOVEMBER, 2025**

## CERTIFICATION

This is to certify that this research titled '**INVESTIGATING THE EFFECT OF AQUEOUS LEAF EXTRACT OF *Camellia sinensis* (GREEN TEA) ON ARSENIC TRIOXIDE INDUCED GASTRIC DAMAGE IN ADULT WISTAR RATS**' for the award of a degree of Bachelor of Science(B.sc) in Anatomy was carried out by **Oyinoluwa Mercy ABIOLA**, under the supervision of Dr E.O Imafidon. All literature used in this study have been acknowledge and properly referenced.

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**(PROJECT SUPERVISOR)**

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**DR. ADAZE .B. ENOGERU**  
**(HEAD OF DEPARTMENT)**

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**DATE**

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**(EXTERNAL EXAMINER)**

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**DATE**

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## **DEDICATION**

I dedicate this work to God Almighty who saw me through my undergraduate studies. Also to my parents Prof Matthew and Mrs. Elizabeth Abiola for their unwavering supports throughout my undergraduate studies and to my siblings for their immense contribution to success of this project.

## **ACKNOWLEDGEMENT**

I would like to express my sincere gratitude to almighty God for his guidance, strength, and wisdom throughout this journey. I also want to appreciate my parents (Mr. and Mrs. Abiola), and my siblings (Praise, Dominion and Micheal) for the love, prayers, financial support which made this work a success. I am also grateful to my supervisor, Dr. Endurance Imafidon whose guidance, encouragement, and support made this work possible. Their valuable insights, patience and advice were useful in the successful completion of this project.

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I am also grateful to myself for the courage to start, patience and determination to see this journey through. The commitment to learning and striving for excellence has been a personal growth, and I take pride in the effort that has brought this work to completion.

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## ABSTRACT

Gastric damage is a common phenomenon in this part of the world, with about 70%–80% of the population having this issue. These have raised major concerns among experts in the medical field. The project “Investigating the Effects of Aqueous Leaf Extract of *Camellia sinensis* on Arsenic-Induced Gastric Damage in Adult Wistar Rats” aims to investigate the protective effects of aqueous leaf of *camellia sinensis* in arsenic-induced stomach damage in adult wistar rats. This study is an experimental research design that involves the use of 30 adult wistar rats, which were randomly divided into six groups (A-F). Group A served as the control group, Group B (Arsenic only), Group C (Arsenic+ Omeprazole), Group D(Arsenic+ low dose of extract), Group E(Arsenic+ high dose of extract) and Group F(Arsenic and left to recover). The rats were given water and feed ad libitum only for 14 days for acclimatization. Thereafter, the five treatment groups were given arsenic trioxide (10mg/kg) orally for 14 days to induce gastric damage. Group B rats were sacrificed for pilot study. Group C received Omeprazole(500mg/kg) for 28 days, Group D was administered low dose of the extract(250mg/kg) for 28 days, Group E received a high dose of the extract 500mg/kg) for 28 days also while the last group was left to recover without administering any extract and thereafter sacrificed by cervical dislocation. The results showed that rats treated with arsenic only had statistically significant decrease in body weight compared to the control group while the rats treated with *camellia sinensis* (250mg/kg) had statistically significant increase in weight compared to the rats in Group B. The rats in Group B had statistically significant decrease in organ weight compared to the control group. Group D had a significant increase in the organ weight compared to Group B. The antioxidant result showed a significant increase in superoxide dismutase, catalase and gluthaione peroxidase level and a significant decrease in malondialdehyde level in Group C, D and E. The histology results showed crater-shaped ulcers in the rats given arsenic toxin only. Normal mucosal lining was seen in the control group as well as in those given arsenic with omeprazole (the standard anti-ulcer drug). The rats given low and high doses of *camellia sinensis* showed pitting mucosal lining, with those given low doses having better ameliorative effects compared to those given high dose. The last group (reversal group), which were given only arsenic and left to recover, showed severe mucosal erosion. In conclusion, the research findings suggest that *camellia sinensis* (green tea) has the potential to mitigate arsenic-induced gastric damage in adult wistar rat.

# CHAPTER ONE

## INTRODUCTION

### BACKGROUND OF STUDY

Foods and water can be polluted with arsenic to some extent. A substantial proportion of the global populations are exposed to arsenic without realizing its negative effects, although often in minute quantity. World Health Organization (WHO, 2022) reported that 140 million people in at least 70 countries drink water contaminated with arsenic above the guideline value and the accumulation of this toxin poses serious risks to human health.

Arsenic is a harmful toxin that shouldn't be found in the human system (Kuivenhoven, 2023). Human exposure to it weakens the immune system (Dangleben *et al.*, 2013) and might have the capacity to negatively affect the gastrointestinal system. The stomach, which is one of the essential organs of the body, can be greatly damaged by arsenic trioxide. The stomach has epithelial linings in its wall that prevent the acidic content from coming in contact with the wall. When the integrity of the epithelial lining is damaged, the acidic content touches the wall of the stomach, causing severe pain.(StatPearls, 2023).

The longer the frequent exposure of stomach to arsenic trioxide the higher the likelihood of gastric damage which may lead to changes in the bowel movement of the gastrointestinal system. This may also galvanize to peptic ulcer disease (PUD) usually located in the stomach and proximal duodenum, referring to acid peptic injury of the digestive tract and its complications, including perforation and bleeding, have been a major threat to the world's population (Sapkota *et al.*, 2022). The global mortality rate was ranked in 2020 by WHO with 44 countries having high risk of death from ulcer. Cambodia came first in the world with highest risk rank of 20.80 (WHO, 2020). Nigeria is among the danger zone of high likelihood of death from ulcer with a rank of 7.03 and number thirty-one in the world. The likelihood

that many Nigerians will be dying from ulcer is high. This is unacceptable. In light of this scenario, the search for natural protective agents has gained attention.

The research question is what can be done to reduce the danger posed by arsenic trioxide. *Camellia sinensis* popularly known as green tea is widely consumed for its numerous health benefits. It is rich in bioactive compounds such as polyphenols, particularly catechins like epigallocatechin gallate (EGCG), which have strong antioxidant and anti-inflammatory properties. These compounds are known to protect cells from oxidative stress and may help in repairing tissue damage. The natural ability of green tea to repair tissue damage has been studied for its potential protective effects against various toxins, including heavy metals and environmental pollutants like arsenic oxide (Chatterjee *et al.*, 2022; Zhao *et al.*, 2018).

## **1.2. JUSTIFICATION**

The incidence of gastric damage is on the rise globally, posing a significant public health concern. Despite the availability of various therapeutic interventions and pharmacological agents aimed at managing and healing gastric damage, complete and effective treatment remains a challenge, with recurrence and side effects being common issues (Lee *et al.*, 2020). This underscores the need for continuous research into alternative and complementary treatment options. *Camellia sinensis*, commonly known as green tea, is renowned for its potent antioxidant and anti-inflammatory properties, which may play a protective and therapeutic role in gastric damage healing (Farzaei *et al.*, 2019). Therefore, this study seeks to investigate the potential effects of *Camellia sinensis* on gastric damages, particularly focusing on its antioxidant and anti-inflammatory mechanisms and how they contribute to gastric damage prevention or healing.

### **1.3. Statement of Problem**

Arsenic exposure continues to pose a serious public health and environmental challenge, especially in many developing nations where enforcement of water quality and food safety regulations is inadequate (WHO, 2023). While antioxidants have been widely investigated for their potential in mitigating arsenic-induced harm, there is limited evidence on how green tea extract specifically influences stomach health in cases of arsenic toxicity (Debnath *et al.*, 2018). Understanding how *Camellia sinensis* counters oxidative stress and inflammatory processes in gastric tissue could pave the way for new therapeutic insights.

### **1.4. AIM OF STUDY**

The aim of this project is to investigate the protective effects aqueous leaf extract of *Camellia sinensis* in an arsenic induced gastric damage in adult wistar Rat.

### **1.5 SPECIFIC OBJECTIVES**

The specific objectives of the study include;

- a. Evaluating the effects of *Camellia sinensis* on body and organ weight induced by arsenic trioxide.
- b. To evaluate the biochemical effects of aqueous *Camellia sinensis* extract on oxidative stress markers in arsenic exposed wistar rats.
- c. Evaluating the therapeutic effects of *Camellia sinensis* on the histopathology changes in arsenic trioxide induced adult wistar rat

### **1.6. SIGNIFICANCE OF THE STUDY**

This research will contribute to scientific knowledge on the protective effects of green tea and therapeutic insights for arsenic-induced gastric damage and offer data that may guide future pharmaceutical and nutraceutical interventions (Khan & Mukhtar, 2019; Akinmoladun *et al.*, 2021).

## **1.7. SCOPE OF THE STUDY**

This study will focus on:

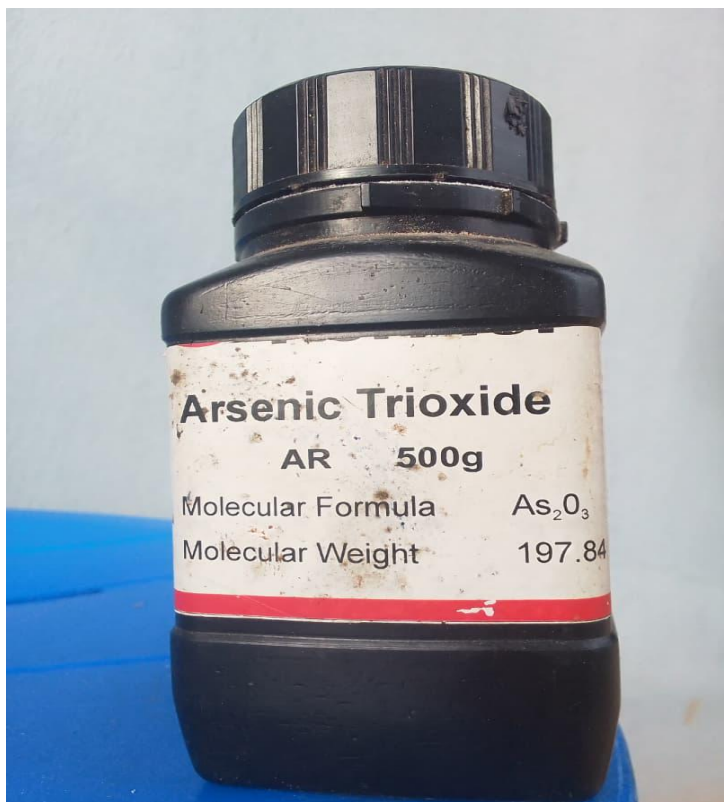
- Using adult female Wistar rats as the experimental model.
- Administering Arsenic Trioxide as the arsenic toxin.
- Evaluating the effects of aqueous green tea extract on oxidative stress, histological alterations, gene expression.
- Conducting biochemical, histopathological, and molecular analyses to determine the protective effects of green tea.

The study is limited to gastric damage and does not extend to other gastrointestinal regions or systemic arsenic toxicity effects.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1. ARSENIC TOXICITY (ARSENIC TRIOXIDE)



Made in china. CAS No: 1327-53-3

#### 2.1 Arsenic Toxicity (Arsenic Trioxide)

Arsenic Trioxide ( $\text{As}_2\text{O}_3$ ) is sometimes referred to as white arsenic and is a form of inorganic arsenic (PubChem, 2025). It occurs as white crystalline solid and has the ability to dissolve slowly in water. It is highly toxic but in modern medicine it is used in controlled very small doses in the treatment of acute promyelocytic leukaemia (Lo-Coco *et al.*, 2013).

##### 2.1.1 Physical properties

It occurs physically as a white crystalline solid and is white in colour. It is odourless.(NCBI, 2023). It exists in two main forms, the cubic and monoclinic form. It has a melting point of 312C and a boiling point of 465C. It has a density of 3.7g/cm<sup>3</sup> and is slightly soluble in cold

water and more soluble in hot water. In water it forms clear solution and it's highly volatile. It is slightly hygroscopic. It is obtained from roasting arsenic containing ore (Haga, Altansukh, & Shibayama, 2018).

### **2.1.2. Chemical properties**

It is amphoteric in nature; it reacts with acids to form arsenious acid and with base to form arsenites. It can be oxidized to arsenic pentoxide (from +3 to +5) (Sharma & Sohn, 2009). It can be reduced by reducing agents to metallic arsenic( Nesbitt *et al.*, 2011). It reacts with sulphides to form arsenic sulphides. It also forms complex salts with alkalis ( Paktunc *et al.*, 2008).

### **2.1.3. SOURCES AND EXPOSURE ROUTES**

The common exposure route of arsenic is ingestion, particularly intake of contaminated water. Other routes include inhalation of arsenic fumes from air and also by skin contact though it is minor (ATSDR, 2007). The principal source of arsenic trioxide in the environment is the oxidation of arsenic bearing sulphide ores during mining and smelting processes. ( Mandal & Suzuki, 2002)

### **2.1.4. MECHANISM OF TOXICITY**

The main mechanism of arsenic trioxide is by the formation of reactive oxygen species (ROS). When arsenic trioxide enters the body it dissolves to form arsenite. It then binds to sulphhydryl groups which allow the arsenites to inactivate enzymes, especially those that are needed for energy production in the mitochondria. The inhibitions of the enzymes would cause the generations of excess reactive oxygen species. The increase of ROS would cause oxidative stress, which would damage lipids, proteins and DNA. (Shi *et al.*, 2004)

### **2.1.5. EFFECTS ON THE GASTROINTESTINAL TRACT**

Exposure of the gastrointestinal tract to arsenic trioxide can lead to severe damage particularly to the stomach, because the stomach is the first point of contact after ingestion. Ingestion of arsenic compounds has been reported to irritate the gastric mucosal lining, causing inflammation (gastritis) and epigastric pain. Arsenic trioxide can also erode the mucosal lining (ATSDR, 2007).

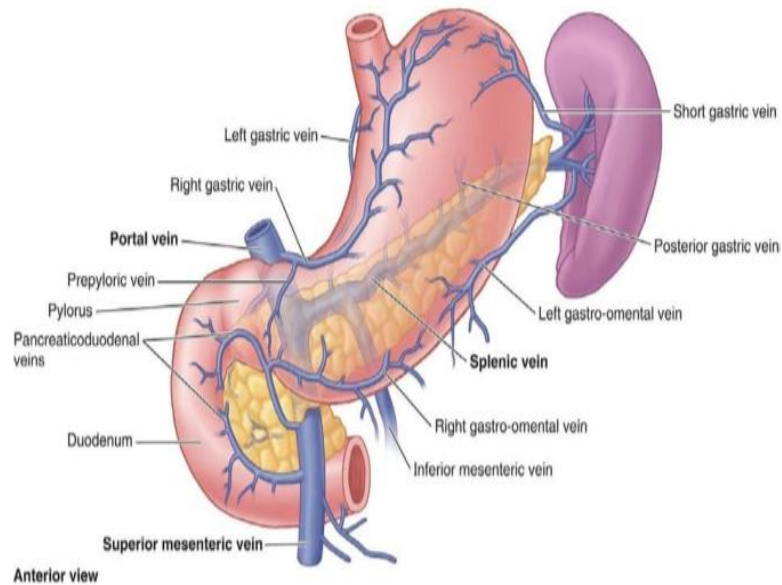
### **2.1.6. COMMON HISTOLOGICAL FINDINGS**

Histological studies of arsenic-exposed stomach commonly show mucosal congestion, desquamation of the surface epithelium. It also shows mucosal and sub mucosal blood vessels that are usually congested and dilated. (ATSDR, 2007).

## **2.2. ORGAN OF STUDY**

### **2.2.1. The Stomach**

The stomach is the expanded part of the alimentary canal that forms the widest and distended part of the digestive tube. It is connected above to the esophagus and below to the small intestine. It acts as a temporary reservoir for food and Its main function is enzymatic digestion (Moore *et al.*, 2018).



**Fig.2.1**

### 2.2.2. Anatomy

The stomach has four parts namely the cardia part, fundus, body and pylorus. The cardia part continues from the esophagus and marks the beginning of the stomach. The fundus is the superior part while the body is the main part and the pylorus continues into the duodenum. The stomach has two curvatures, the greater and lesser curvature. The right, short concave border is the lesser curvature and the left, longer convex border is the greater curvature. At the inferior end of the lesser curvature, there is a notch called the angularis incisor which marks the junction of the body and pyloric part of the stomach (Drake *et al.*, 2019).

The stomach is an intraperitoneal organ, which implies that it is covered completely by peritoneum except parts where its blood vessels run along its curvature. The peritoneum forms the greater and lesser omentum. The greater omentum falls from the greater curvature as a double layered membrane and inserts into the transverse colon. The lesser omentum falls from the lesser curvature and inserts into the superior part of the duodenum. The stomach is related anteriorly with the anterior abdominal wall, the diaphragm and the left lobe of the liver. Posteriorly it is related with the structures that make up the stomach bed, pancreas and

omental bursa. Inferiorly and laterally, the stomach is related to the transverse colon (Moore *et al.*, 2018).

### **Location and Position**

The stomach is located in the upper left quadrant of the abdomen and it occupies the left hypochondrium, epigastric and umbilicus region. The stomach extends obliquely from the left hypochondrium to the epigastric region. It lies below the left costal cartilage and ribs (Netter, 2018).

### **Size and Shape**

The stomach is J shaped when empty but when distended it loses its shape. The stomach has about 50 ml size capacity (Drake *et al.*, 2019).

### **Regions of the stomach**

As earlier stated, the stomach has four parts which are the cardia end, fundus, body and pylorus. The body is the main part where digestion takes place, the cardia end marks the beginning of the stomach, the fundus is the most superior part and the pylorus continues into the small intestine (Netter, 2018).

### **Curvatures of the Stomach**

The stomach has two curvature, the lesser and greater curvature which are on the right and left side of the stomach respectively. The lesser curvature forms the short and convex border of the stomach while the greater curvature forms the longer and concave border of the stomach (Moore *et al.*, 2018).

### **Blood supply of the stomach**

The stomach receives blood supply from branches of the celiac trunk and splenic artery. The veins of the stomach correspond with the arteries and eventually drain into portal venous system through the superior mesenteric vein and splenic vein (Drake *et al.*, 2019).

### **Innervation of the stomach**

The para-sympathetic nerve supply of the stomach is from the anterior and posterior vagal trunk and their branches. While that of the sympathetic nerve supply is from the T6-T9 spinal segment, passing through the greater splanchnic nerve and celiac plexus (Netter, 2018).

### **2.2.3. Clinical Significance**

The stomach is susceptible to various disorders, often linked to environmental, microbial, and chemical factors:

- 1• Gastritis: This is the Inflammation of the gastric mucosa, that is often caused by *Helicobacter pylori*, NSAIDs, alcohol, or stress (Moore *et al.*, 2018).
- 2• Gastric Ulcer: This is erosion of the stomach lining up to the muscularis mucosa majorly because of the imbalance between protective and aggressive factors (Moore *et al.*, 2018).
- 3• Gastric Cancer: Also called cancer of the stomach, it occurs when there is genetic mutation or chronic inflammation leading to formation of Malignant tumors (Netter, 2018).
- 4• Gastroesophageal Reflux Disease (GERD): It occurs when gastric contents reflux into the esophagus due to lower esophageal sphincter dysfunction (Netter, 2018).

### **2.2.4. HISTOLOGY OF THE STOMACH**

The wall of the stomach is made up of four layers, which are the mucosa layer, the sub mucosa layer, the muscularis layer and the serosa layer (Drake *et al.*, 2019).

#### **Mucosa layer**

This is the innermost layer and it comprises of epithelial lining, glands, cells, laminal propria and muscularis mucosa. The muscularis mucosa separates it from the sub mucosa layer (Moore *et al.*, 2018).

The epithelial lining of the stomach is simple columnar ciliated epithelium. The mucosa of the stomach shows invagination called gastric pits and gastric glands open into them (Netter, 2018).

There are various glands in the mucosa layer of the stomach:

1. Cardia gland: They are located in the cardia part of the stomach, hence their name. These glands are either simple tubular or compound tubular-alveolar. They are mucus secreting glands and oxyntic or peptic cells may be present in them (Moore *et al.*, 2018).
2. Main gastric glands: They are present all over the stomach except the pyloric region. These glands are either simple or branched tubular gland and they open into gastric pits (Drake *et al.*, 2019).
3. Pyloric glands: They are short, simple or tubular glands that are coiled. They open into the gastric pits and are lined by mucus secreting cells. Sometimes, oxyntic or argentafin cells may be present in them and they secrete gastrin (Netter, 2018).

The cells of the stomach are as follows:

- 1• Chief cells: They are cuboid cells that are also called peptic cells. They are the most numerous cells and are especially numerous in the basal part of the glands. They secrete digestive enzymes of the stomach and pepsin that breakdown protein into small peptide (Drake *et al.*, 2019).
- 2• Oxyntic cells: They are large ovoid cells with a large central nucleus. They are also known as parietal cells. They occur singly among the chief cells. They secrete hydrochloric acid and intrinsic factor that combines with vitamin B12 to help in the normal function of erythrocytes (Netter, 2018).
- 3• Mucous neck cells: They are found in the neck of glands and are mucus-secreting cells (Moore *et al.*, 2018).
- 4• Endocrine glands:

### **Submucosa**

This is the second layer of the stomach and it consists of dense, irregular collagenous tissues. It contains fibroblast cells, mast cells and lymphoid tissue. It also contains the Meissner plexus and blood vessels that supply and drain the mucosa (Netter, 2018).

### **Muscularis External**

This is the third layer of the stomach from inside out and it consists of three layers of muscles arranged transversely. They include an incomplete oblique muscle, a thick middle circular muscle and an outer longitudinal muscle. Between the circular and longitudinal muscle, the Auerbach myenteric plexus is found. The circular muscle forms the pyloric splinter (Moore *et al.*, 2018).

### **Serosa (Drake *et al.*, 2019)**

This is the most outermost layer of the stomach and it helps to provide structural support and contains blood vessels. It is covered by thin squamous epithelium and it helps to reduce friction between the stomach and other organs. ( Moore *et al.*, 2018).

### **2.2.5. Development**

The stomach develops from the caudal part of the foregut as a fusiform dilation. This dilation has an anterior and posterior border and also left and right surfaces. The stomach then rotates 90° clockwise so that the left surface faces anteriorly while the right surfaces faces posteriorly (Moore *et al.*, 2018). During the rotation, the posterior border grows faster than the anterior border, therefore forming the greater and lesser curvature respectively (Saddler, 2020).

The stomach also rotates along its anterior–posterior axis, so that the distal or pyloric end moves to the right and the proximal or cardia end moves to the left. The 90° degree rotation moves the dorsal mesogastrium to the left side, thereby forming the lesser sac or omental bursa (Langman’s Medical Embryology, 2021).

### **2.2.6. FUNCTION OF THE STOMACH**

The stomach performs several functions to ensure the proper breakdown of food and preparation of food for nutrients absorption by the intestine (Drake *et al.*, 2019).

#### **Mechanical digestion**

The stomach acts as a temporary reservoir for foods and thereby controlling the release of food into the intestine for absorption. Food is broken down mechanically by muscular peristalsis which is done by contractions of the smooth muscles in the stomach (Moore *et al.*, 2018).

#### **Chemical digestion**

The stomach secretes hydrochloric acid which lowers the pH of the stomach thereby creating an acidic environment in the stomach. This helps in activation digestive enzymes and denaturing proteins. The stomach also secretes pepsinogen which when activated into pepsin breaks down protein into small peptides (Tortora & Derrickson, 2022).

#### **Secretory function**

The stomach secretes substances that help in digestion and absorption. Examples include hydrochloric acid (HCL), pepsinogen and gastric lipase (Netter, 2018).

#### **Hormonal function**

The cells in the stomach secrete hormones that help in regulation of digestion of food. Examples include gastrin which stimulates the release of gastric acid. Others are ghrelin which is known as hunger hormones and it stimulates hunger (Drake *et al.*, 2019).

#### **Absorption function**

Although most absorption functions are performed by the small intestine, the stomach plays a little role in absorption. It absorbs water in small amount and also absorbs alcohol. The reason for the little absorption in the stomach is because of its thick mucosa wall (Moore *et al.*, 2018).

### 2.2.7. EVALUATION CRITERIA IN WISTAR RATS

Experimental studies involving Wistar rats use several criteria to evaluate stomach integrity, including gastric pits, mucus glands, and epithelial cell morphology. Histopathological scoring systems are used to assess degrees of inflammation, necrosis, and mucosal erosion. These evaluations are vital in toxicology studies to determine the severity of chemical-induced intestinal injury and the potential therapeutic effects of test substances (Ali *et al.*, 2015)

### 2.3. PLANT OF STUDY

*Camellia sinensis* is a popular plant that originated from south Asia but is cultivated wide across the world (Meegahakumbura *et al.*, 2018 is). It is the source of green tea, black tea and white tea. The *Camellia sinensis* is known to have numerous health benefits due to its antioxidant properties and is popularly taken as a beverage (Musial *et al.*, 2020). Its leaves and stems are of great benefits as they are the source of different products.



**Fig.1 *Camellia sinensis* (Green Tea) Leaf**

Source: Healthline.com

### 2.3.1 DESCRIPTION

Country	Dialect
India	Ccha
China	Cha
Russia	Chai
Italy	Te
England	Tea plant
United States	Tea

**Fig.2 Common sinensis name**

Camellia sinensis was first cultivated in China and south Asia but today it is cultivated across the world in tropical and sub tropical regions ( Meegahakumbura *et al.*, 2018). It is a small tree that is trimmer to below 2 meters if cultivated for its leaves (Mondal *et al.*, 2022). Green Tea (camellia sinensis) is widely recognized for its leaves which possess great properties which have been attributed to its polyphenol content (Shirakami & Shimizu, 2018).

#### **Leaves**

The leaves of camellia sinensis are short stalked and varying length of 5-10cm long and 2.5-4cm broad. The young, light green leaf are preferred for tea production. On their underside they have white hairs. The older leaves are deep green (Mondal *et al.*, 2022).

#### **Flower**

Its flowers are yellow-white in colour. They are solitary or found in clusters and they have 5-7 petals. The Flowers of camellia sinensis bear numerous stamens with yellow anther and produce woody capsules (Naing *et al.*, 2019).

#### **Location**

Camellia sinensis from the family-Theaceae originated from Southeast China and gradually expanded into many tropical and sub-tropical countries. It is grown in several countries

Worldwide . It grows best in tropical and subtropical areas with adequate rainfall, good drainage and slightly acidic soil.( Wang *et al.*, 2022).

### **Fruits**

They are typically globuse and are about 1.5 - 3cm in length. The fruits are dry, woody and dehiscent. The capsule of the fruits usually contains 1-2 seeds although they might be fewer.(GlobinMed, n.d.; University of Mandalay Research Journal, 2020).

### **Seed**

The seeds of *Camellia sinensis* are dark brown in colour and occur inside the capsule of the fruit. They are processed to produce camellia seed oil, which is used in food and cosmetic applications (INCI Beauty, n.d.). The seed oil also contains catechins (Zeng *et al.*, 2019).

## **2.3.2. SCIENTIFIC CLASSIFICATION**

Here is the taxonomy for *Camellia sinensis* (Green Tea).

<b>Kingdom</b>	<b>Plantae</b>
<b>Division</b>	<b>Magnoliophyta</b>
<b>Class</b>	<b>Magnoliopsida</b>
<b>Order</b>	<b>Ericales</b>
<b>Family</b>	<b>Theaceae</b>
<b>Genus</b>	<b>Camellia</b>
<b>Species</b>	<b>C.sinensis</b>

**Fig.2 . Taxonomy *Camellia sinensis* (Green Tea).**

## **2.3.3. PHYTOCHEMICAL PROPERTIES**

*Camellia sinensis* is the source of green, black, and oolong teas, is popular for its rich phytochemical properties, which underpins its numerous health benefits (Musial *et al.*, 2020).

1• Polyphenol: It makes up about one third of camellia sinensis phytochemical properties, making it the most abundant. The polyphenol most active and abundant biological compound is the catechin. The catechins are thought to be the factor responsible for the camellia sinensis health benefits. There are various types of catechins. The major ones include; the epicatechin gallate(ECG), epicatechin(EC), epigallocatechin(EGC) and epigallocatechin gallate (EGCG). The epigallocatechin gallate(EGCG) is the most abundant and active of the catechins (Shirakami & Shimizu, 2018).

2• Alkaloids: It includes caffeine, theobromine and theophylline. Caffeine is the most abundant of them and it helps in increasing alertness and improving cognitive functions (Wang *et al.*, 2022).

3• Amino Acids: It is about 1-4% of the dry weight of camellia sinensis. The most prevalent amino acid is the L-theanine, which comprises approximately 60–70% of the total free amino acids in tea. L-theanine is associated with relaxation and stress reduction, counteracting the stimulating effects of caffeine (Yang *et al.*, 2022).

4• Vitamin and minerals: Vitamin presents in camellia sinensis are vitamins A, C and E. While the minerals present include manganese, fluoride, zinc, potassium and magnesium (Bashiru *et al.*, 2019 ).

5• Saponins: They exhibit antioxidant and anti-inflammatory effects (Fan *et al.*, 2021).

#### **2.3.4. MEDICINAL AND PHARMACOLOGICAL EFFECTS OF *Camellia sinensis* (Green Tea)**

*Camellia sinensis*, commonly referred to as green tea, has long been recognized for its health promoting properties. Modern pharmacological research attributes these benefits to its rich content of polyphenols and catechins, particularly epigallocatechin-3-gallate (EGCG), which is highly bioactive (Musial *et al.*, 2020). However little research has been done in regards to

its effect on the gastrointestinal tract, particularly the stomach. Here are some of the health benefits of *Camellia sinensis*.

- **Antioxidant Activity**

Green Tea is long known for its antioxidant properties which helps in reducing oxidative stress. These antioxidant properties is primarily due to the presence of catechins, the ECG, EC, EGCG and EGC. The catechinshelps in neutralizing radicals and reducing oxidative stress ( Shirakami & Shimizu, 2018). Studies on animals have shown that green tea reduces oxidative stress in the liver and kidney( Thompson *et al.*, 2012).

- **Cardiovascular effects**

Green Tea influences cardiovascular health by improving lipid metabolism, vascular functions and blood pressure regulation. The catechins in the green tea stops the oxidation of low density lipid(LDL), ensures the bioavailability of nitric oxide(NO) for vasodilation and prevents platelet aggregation (Musial *et al.*, 2020, Babu *et al.*, 2008).

- **Anti-cancer potential**

In recent studies green tea is seen to exhibit chemoprotective and therapeutic effects in cancer. Catechins particularly ECGC inhibits tumorigenesis by inhibiting oncogens and activating tumour suppressor and by inducing apoptosis through caspase pathway (Ahmed *et al.*, 2019).

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1. ETHICAL APPROVAL**

An ethical proposal was conducted, which was reviewed and approved by the college Research Ethics Committee of school of Basic Medical Sciences, University of Benin. With this approval number CMS/REC/2025/780.

#### **3.2. REAGENTS AND CHEMICALS**

- Distilled water
- Reagent 1: solution of camellia sinensis leaf extract
- Reagent 2: solution of omeprazole

#### **EQUIPMENTS**

Refrigerator, Orogastric tube, Cotton wool, weighing balance (Wetter PGN), Masking tape, Plastic basket, Plastic cages, Measuring cylinder (Pyrex, USA, 500ml), Sterile plain containers, Plain sample containers, Surgical latex gloves, Dissecting set.

#### **3.3. COLLECTION OF SEED MATERIAL**

##### **REAGENT 1: Camellia sinensis leaf**

Processed Camellia sinensis leaf was gotten from the supermarket, Stop 2 shop supermarket, Benin city, Edo state.



Manufactured in Nigeria , NAFDAC REG NO: 08-8177

### **3.4. EXTRACT PREPARATION**

**REAGENT 1:** *Camellia sinensis*

Aqueous extraction of green tea leaf was performed using a freeze-drying technique. Green tea were first air-dried at room temperature, and then milled into a fine powder using an electric blender. Five hundred grams (500g) of the powdered sample were soaked in 2 liters of distilled water for 24 hours. The mixture was filtered using white filter paper to separate the residue from the liquid extract. The resulting filtrate was concentrated in the Department of Anatomy, Faculty of Basic Medical Science, University of Benin.

### **3.5. EXPERIMENTAL ANIMALS**

Wistar rats were sourced in Anatomy animal house and taken care of in the Animal House, Department of Anatomy, University of Benin, Benin City. Wistar rats were kept in polypropylene cages at room temperature. The acclimatization of the rats was done 2 weeks before experiment initiation. The animals received free access to standard animal feeds (Topfeeds grower mash) and tap water ad libitum. Experiment procedures were in accordance

with the guide for laboratory animal care and use (National Research Council of the National Academics, 2011).

### 3.6. ANIMAL CARE & MANAGEMENT

A total of 30 adult Wistar rats were used for the experiment. The rats were randomly selected, weighed and divided into six groups. They were fed with appropriate amount of feed and water for the period of 42 days.

### 3.7. EXPERIMENTAL DESIGN

Thirty (30) adult Wistar rats were used for this study. After the period of acclimatization, the animals were randomly assigned into six (6) groups (A, B, C, D, E, and F) of five (5) rats each.

GROUP	REGIMEN	DURATION
Group A	Control	42 days
Group B	10mg/kg Arsenic trioxide only	14 days
Group C	10mg/kg body weight of Arsenic trioxide for 14 days + 500mg/kg body weight of standard drugs (omeprazole) for 28 days	42 days
Group D	10mg/kg body weight of Arsenic trioxide for 14 days + 250mg/kg aqueous leaf extract of Camellia Sinensis for 28 days	42 days
Group E	10mg/kg body weight of Arsenic trioxide for 14 days + 500mg/kg of aqueous leaf extract of Camellia Sinensis for 28 days	42 days
Group F	10mg/kg Arsenic trioxide only for 14 days and allowed to recover	42 days

All administrations were given orally with the help of an orogastric tube, throughout the entire study period of forty-two (42) days.

### **3.8. ANIMAL SACRIFICING PROCEDURE**

At the termination of experimental duration, the animals were grossly inspected for general physical characteristics, and weighed on a top loader weighing balance. The animals were sacrificed by cervical dislocation; midline incision was made through the ventral wall of the rat abdomen. The stomach was removed and fixed in 1% formal saline for histological analysis.

### **3.9. HISTOLOGICAL PROCEDURE**

#### **EXAMINATION**

Stomach tissues were excised at once from each group and preserved in 10% Buffered formalin saline solution and prepared for histological analysis using haematoxylin and eosin stains, photomicrographs were taken with the assistance of digital microscopic eye piece device.

### **3.10. PREPARATION OF TISSUE FOR MICROSCOPIC EXAMINATION**

The process of preparation of stomach tissue for histological examinations were done in different stages, which includes; fixation, tissue processing, sectioning & mounting, staining and photomicrography.

### **3.11. TISSUE PROCESSING**

The tissues were sectioned to about 3-5mm thick sections and processed according to method of Drury and Wallington (1980). They were dehydrated for one hour each at room temperature in increasing grades of ethanol: 70% ethanol, 90% ethanol, Absolute ethanol I and Absolute ethanol II. The dehydrated tissues were cleared at room temperature in two changes of Xylene for one hour in each change. The tissues were immersed in two successive

changes of molten paraffin wax multi block plastic embedding mould. Paraffin blocked tissues were sliced and placed on wooden chunk for sectioning in a rotatory microtome. Sections of 5 mm thickness were sectioned from tissue blocks in a rotatory microtome (Bright B5143, Huntington, England). The sections were transferred to water bath at (40°C) to enable spreading of folded ribbons of sections. The sections were mounted on new clean glass slide afterwards and they were dried at 40°C using a slide drier to enhance adhesion of the sections of the slides. Drury and Wallington (1980) staining protocols of Hematoxylin and Eosin was used.

### **3.12. HEMATOXYLIN AND EOSIN STAINING**

Paraffin wax is poorly permeable to stain, so section was deparaffinized in two changes of Xylene for two minutes in each change. Xylene was removed because it does not mix freely with aqueous solution and low grades of alcohol used in the procedure of preparing stains. Thus, sections were then hydrated through a series of descending grades of alcohol until water is used. The aim of this step is to ready the tissue for staining with dye in an aqueous solvent. H&E procedures as per Drury and Wallington (1980) and Scheehan and Hrapchak (1980) were utilized as follows:

- Sections were dewaxed in two changes of Xylene for two minutes in each changes;
- Sections were rehydrate in descending grades of alcohol (Absolute I, Absolute II, 95%, 90%, 70%, and 50% ethanol) for two minutes each;
- Sections were rinsed in distilled water for three minutes;
- Sections were stained in Hematoxylin for 15-20 minutes;
- Excess Hematoxylin stain were removed by rinsing well in running tap water for two or three minutes (sections would be examined microscopically at this stage to confirm sufficient degree of staining);

- Sections were differentiated in acid alcohol (0.5% HCL in 70% ethanol) for two or three seconds. Hematoxylin blue stain became red as a result of the action of acid;
- Sections were washed well under running tap water for 10-15 minutes to remove excess differentiator and to bring back the blue colour of the sections as observed by naked eye;
- Sections were counter stained in 1% aqueous eosin for two to four minutes;
- Excess stain were washed off in running tap water and examined under microscope;
- Sections were dehydrated quickly in ascending grades of ethanol (50% through Absolute ethanol), cleared in Xylene and mounted in a synthetic resin medium (DPX) using clean glass cover slip.

### **3.13. PHOTOMICROGRAPHY**

Histological sections were examined under Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at various magnifications i.e 100X and 400X.

### **3.14. STATISTICAL ANALYSIS**

Data were statistically analyzed using Graphpad Prism and the relevant analytical values were obtained. Statistical significance was determined by means of one-way analysis of variance, followed by Turkey's multiple comparison post-hoc, and data were presented as Mean  $\pm$  Standard Error of Mean (SEM). The post-hoc test was carried out for all groups compared with control. Values of  $P < 0.05$  was considered statistically significant. The statistical values obtained was converted into graphical representation in form of bar charts.

## CHAPTER FOUR

### RESULTS

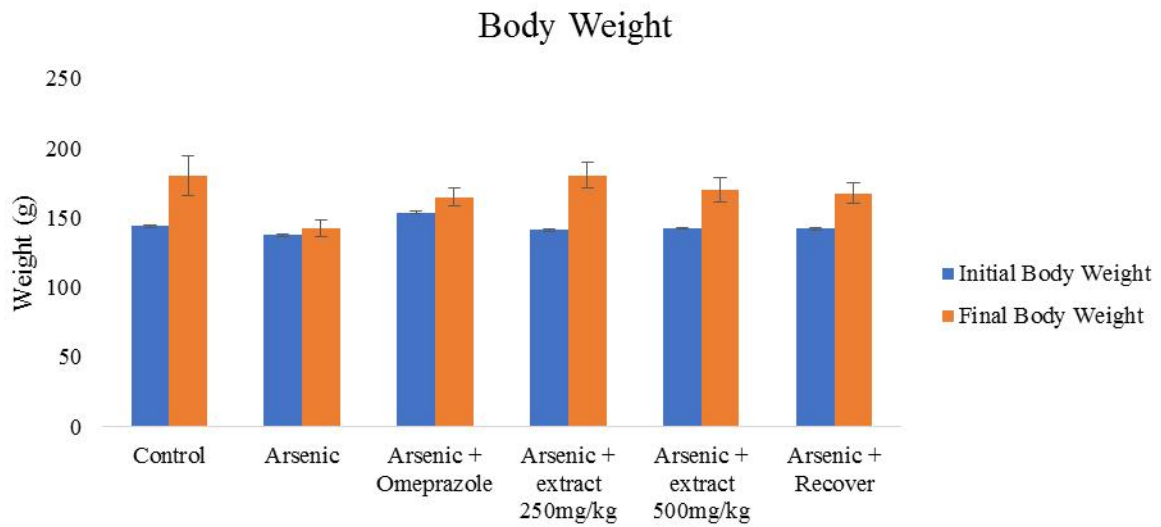


Figure 4.1: Chart showing initial and final body weights across the experimental groups. Values are given as Mean  $\pm$ SEM. \*  $p < 0.05$  compared with control group; #  $p < 0.05$  compared with Arsenic only group.

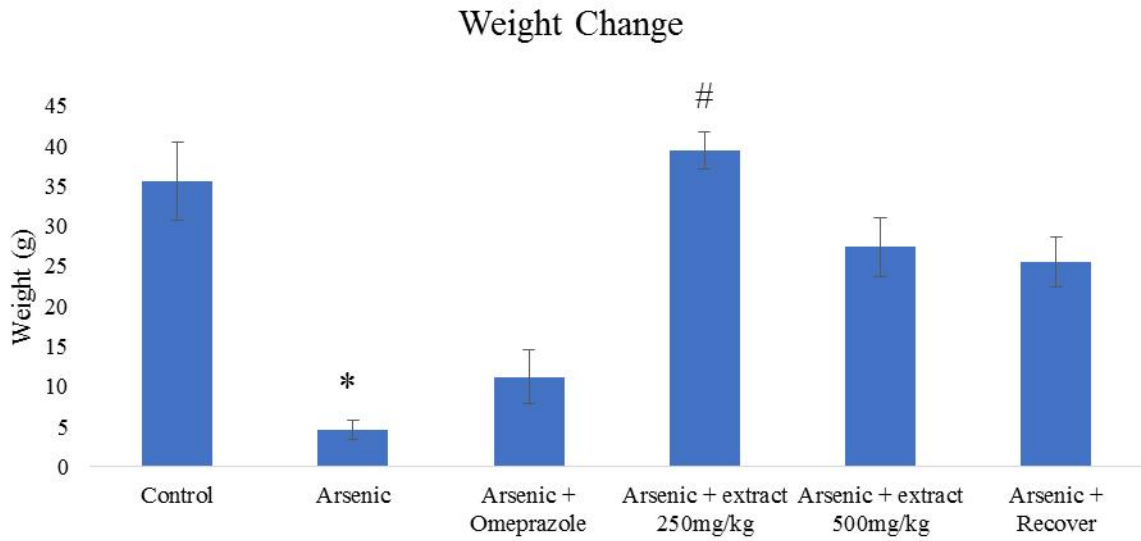


Figure 4.2: Chart showing body weight changes across the experimental groups. Values are given as Mean  $\pm$ SEM. \*  $p < 0.05$  compared with control group; #  $p < 0.05$  compared with Arsenic only group.

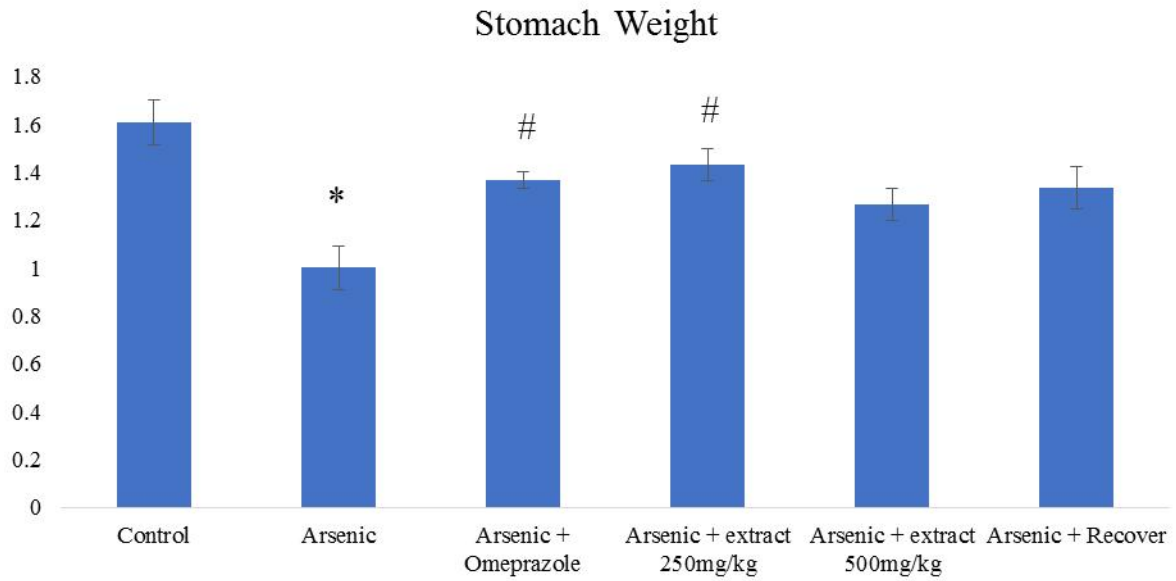


Figure 4.3: Chart showing stomach weight across the experimental groups. Values are given as Mean  $\pm$ SEM. \*  $p < 0.05$  compared with control group; #  $p < 0.05$  compared with Arsenic only group.

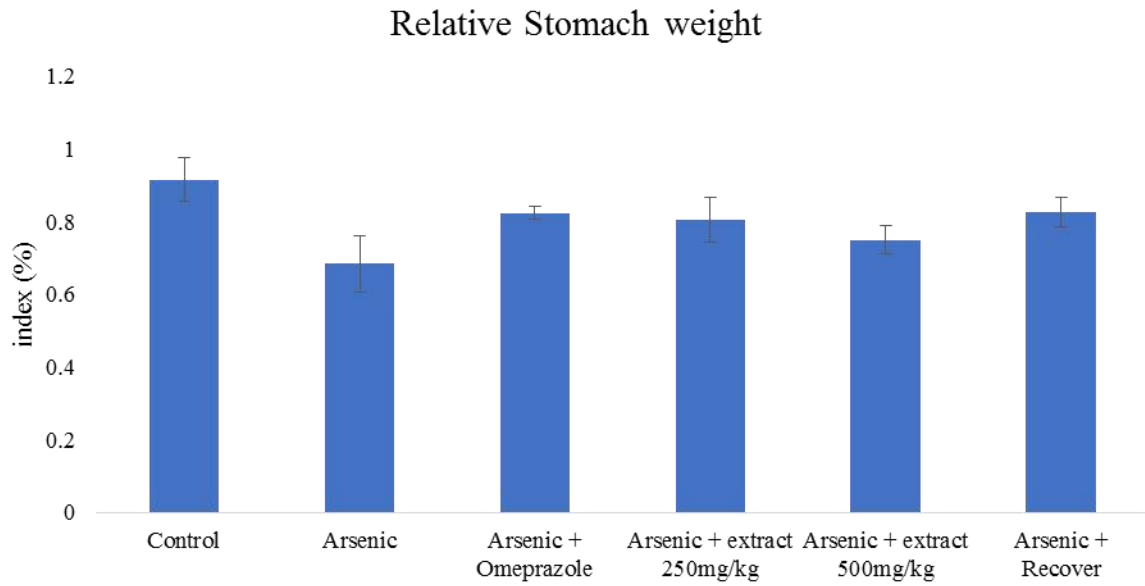


Figure 4.4: Chart showing relative stomach weight across the experimental groups. Values are given as Mean  $\pm$ SEM. \*  $p < 0.05$  compared with control group; #  $p < 0.05$  compared with Arsenic only group

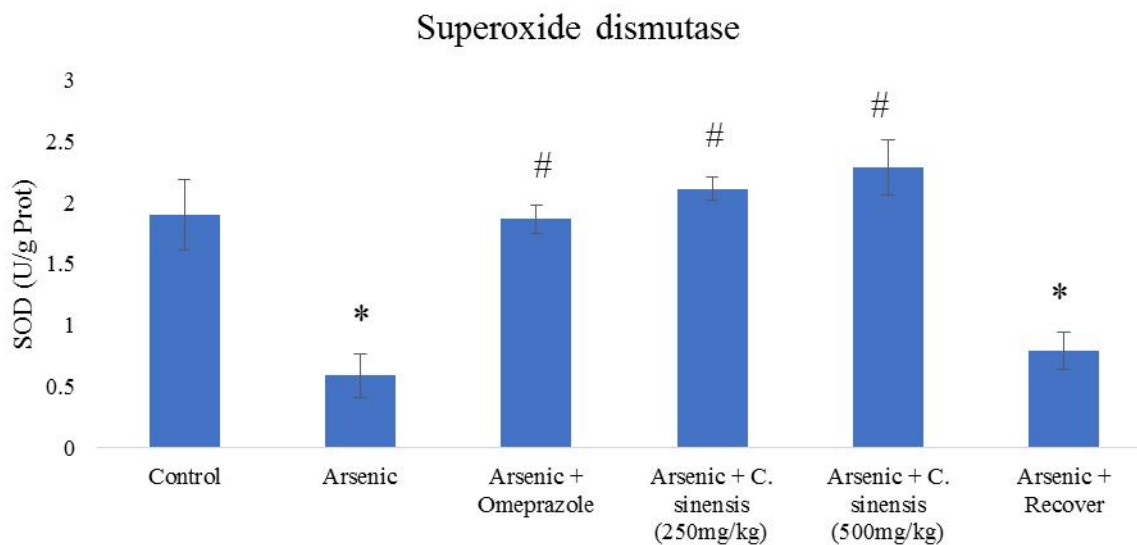


Figure 4.5: Chart showing superoxide dismutase activity across the experimental groups. Values are given as Mean  $\pm$ SEM. \*  $p < 0.05$  compared with control group; #  $p < 0.05$  compared with Arsenic only group

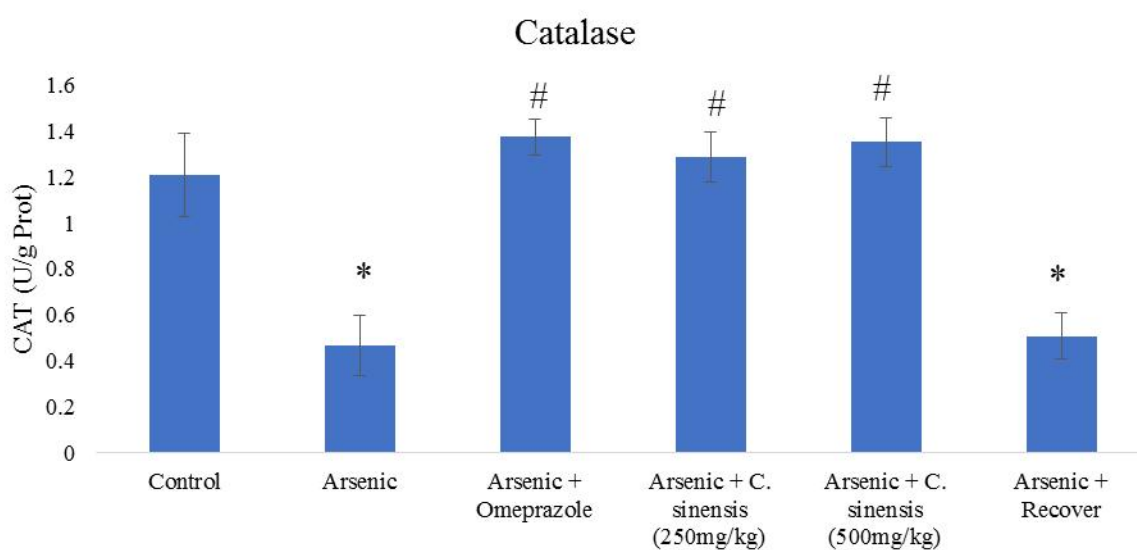


Figure 4.6: Chart showing Catalase activity across the experimental groups. Values are given as Mean  $\pm$ SEM. \*  $p < 0.05$  compared with control group; #  $p < 0.05$  compared with Arsenic only group.

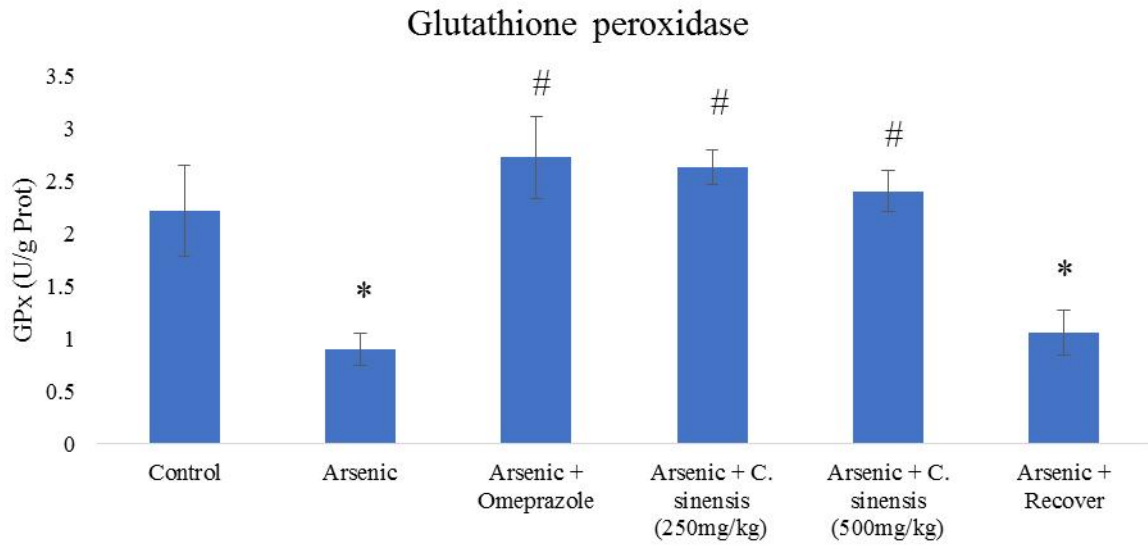


Figure 4.7: Chart showing Glutathione peroxidase activity across the experimental groups. Values are given as Mean  $\pm$ SEM. \*  $p < 0.05$  compared with control group; #  $p < 0.05$  compared with Arsenic only group.

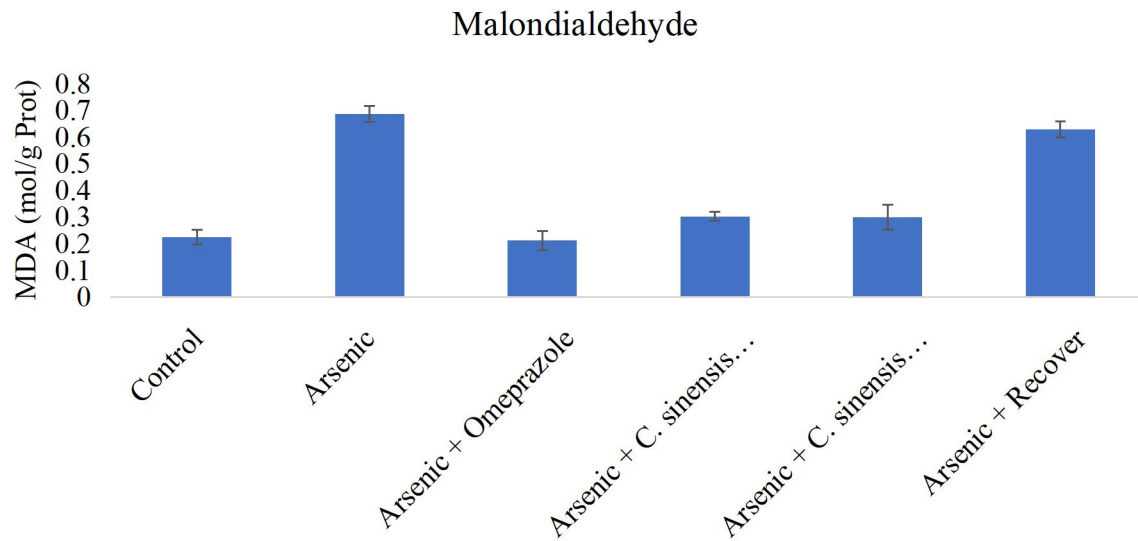


Figure 4.8: Chart showing Malondialdehyde concentration across the experimental groups. Values are given as Mean  $\pm$ SEM. \*  $p < 0.05$  compared with control group; #  $p < 0.05$  compared with Arsenic only group.

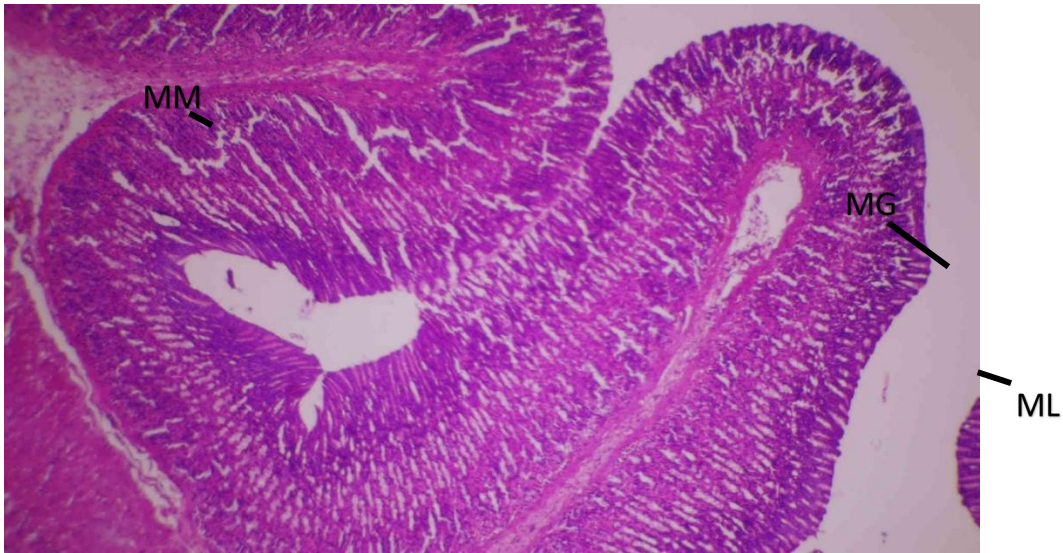


Plate 1. Rat stomach, control, composed of: normal architecture: pitting mucosal lining (ML), mucosal glands (MG), muscularis mucosa (MM): H&E 40 X

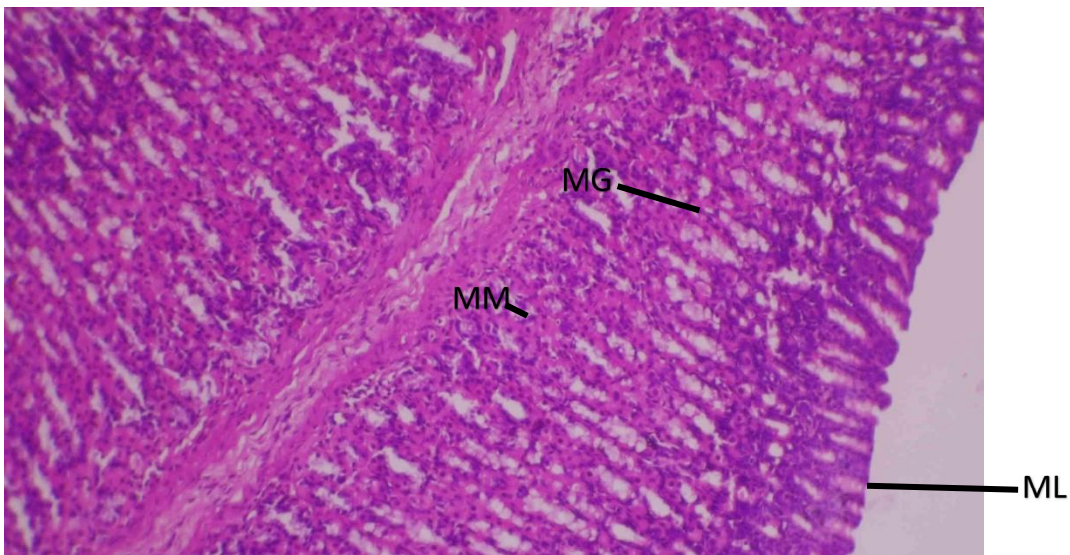


Plate 2. Rat stomach, control, composed of: normal architecture: pitting mucosal lining (ML), mucosal glands (MG), muscularis mucosa (MM) : H&E 100 X

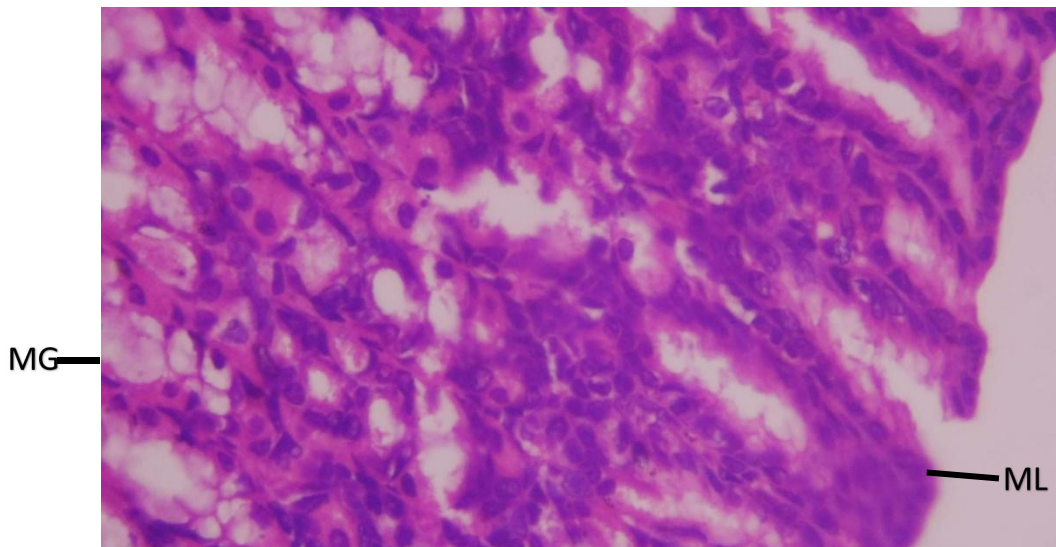


Plate 3. Rat stomach, control, composed of: normal architecture: pitting mucosal lining (ML) and mucosal glands (MG) :H&E 400 X

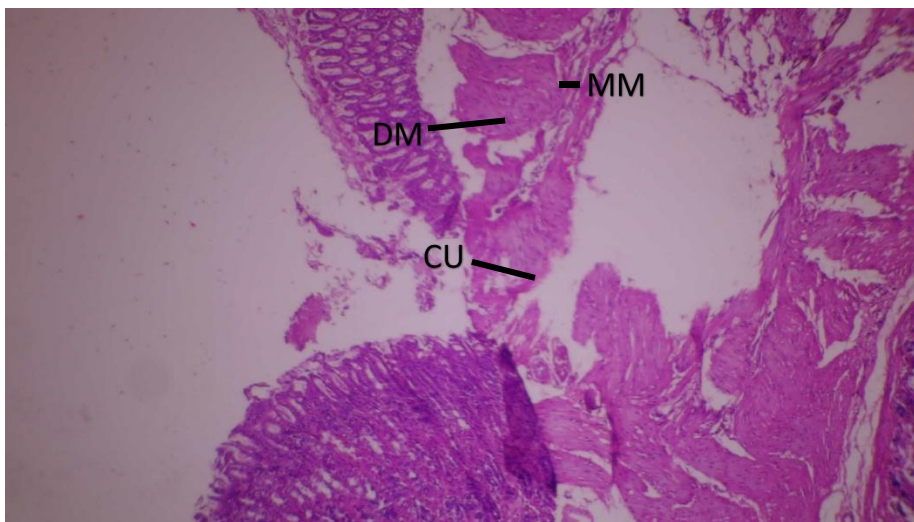


Plate 4. Rat stomach given Arsenic only show: crater-shaped ulcer (CU), muscularis mucosa (MM) and devitalized mucosal tissue (DM): H&E 40 X

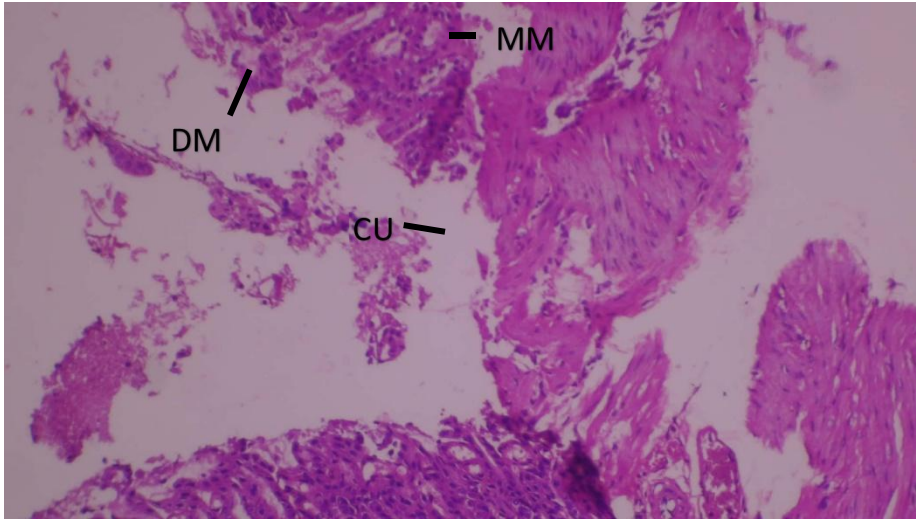


Plate 5. Rat stomach given Arsenic only show: crater-shaped ulcer (CU), muscularis mucosa (MM) and devitalized mucosal tissue (DM) : H&E 100 X

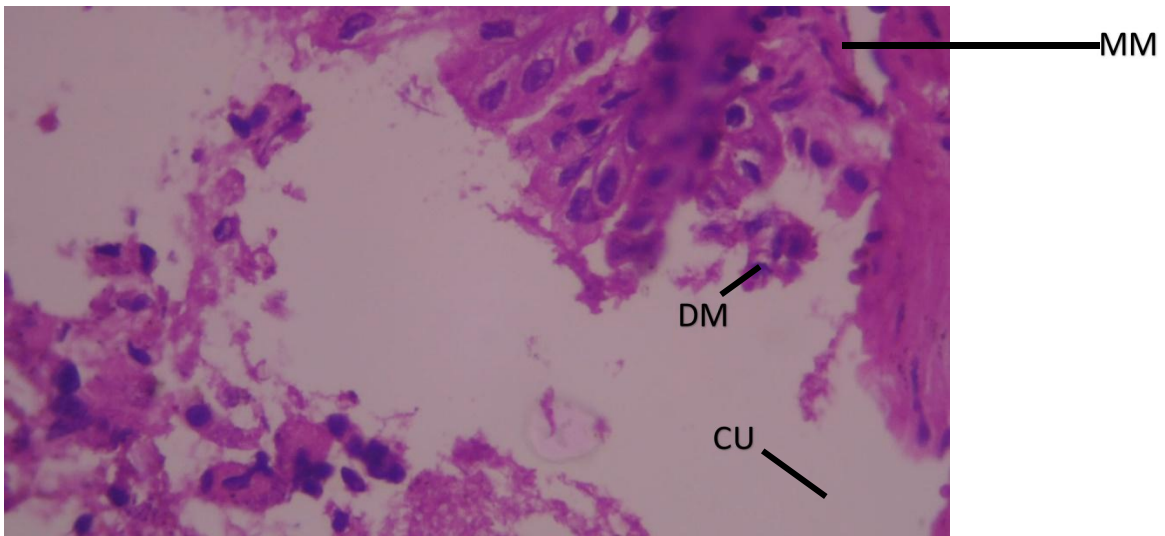


Plate 6. Rat stomach given Arsenic only show: crater-shaped ulcer (CU), muscularis mucosa (MM) and devitalized mucosal tissue (DM): H&E 400 X

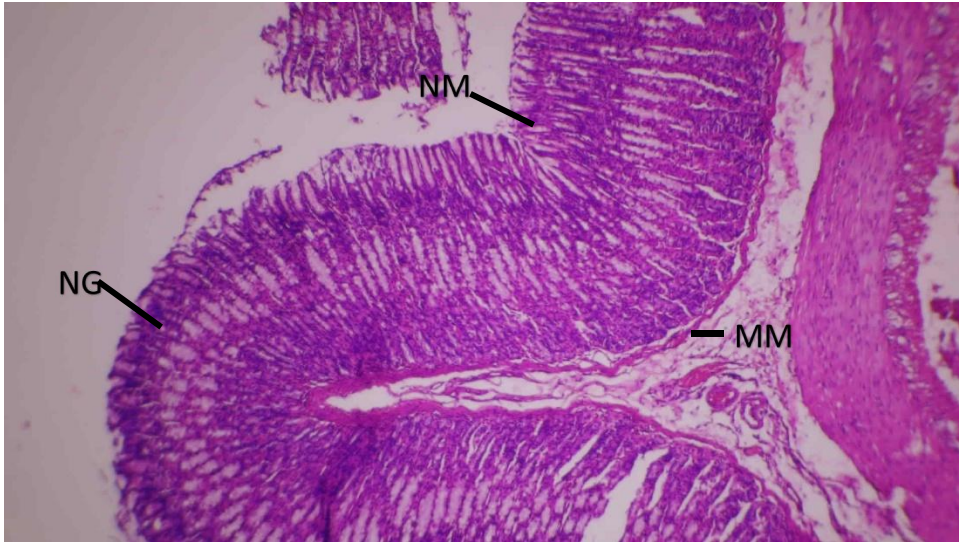


Plate 7. Rat stomach given Arsenic + Omeprazole show: normal mucosal Membrane (NM), mucosal glands (NG) and muscularis mucosa (MM): H&E 40 X

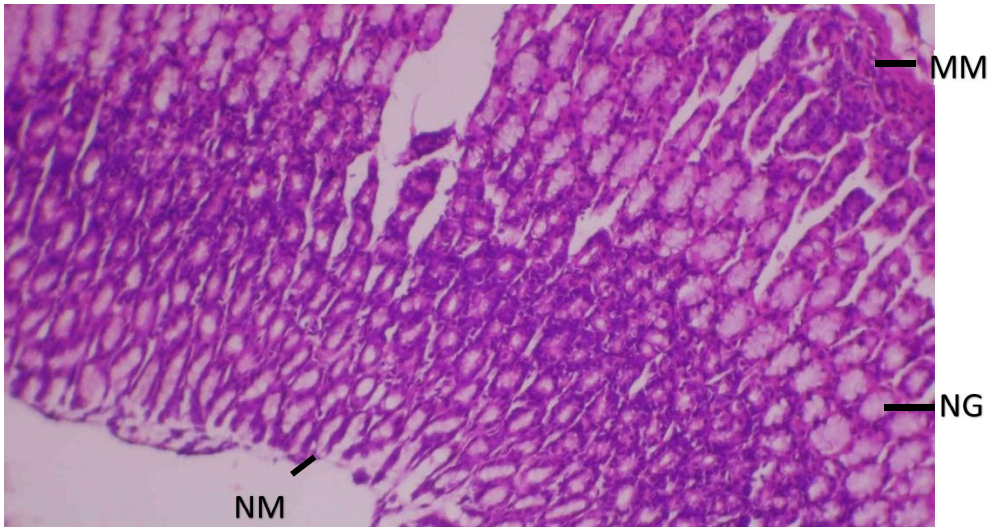


Plate 8. Rat stomach given Arsenic + Omeprazole show: normal mucosal Membrane (NM), mucosal glands (NG) and muscularis mucosa (MM) : H&E 100 X

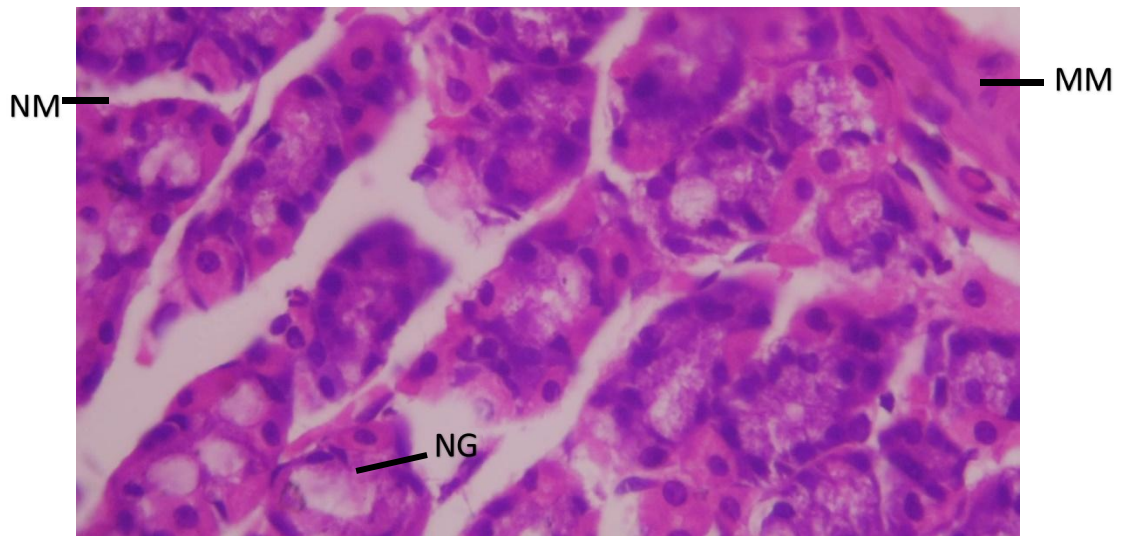


Plate 9. Rat stomach given Arsenic + Omeprazole show: normal mucosa (NM), mucosal glands (NG) and muscularis mucosa (MM) :H&E 400 X



Plate 10. Rat stomach given Arsenic + low dose extract show: normal architecture: pitting mucosal lining (PM), mucosal glands (MG) and muscularis mucosa (MM): H&E 40 X

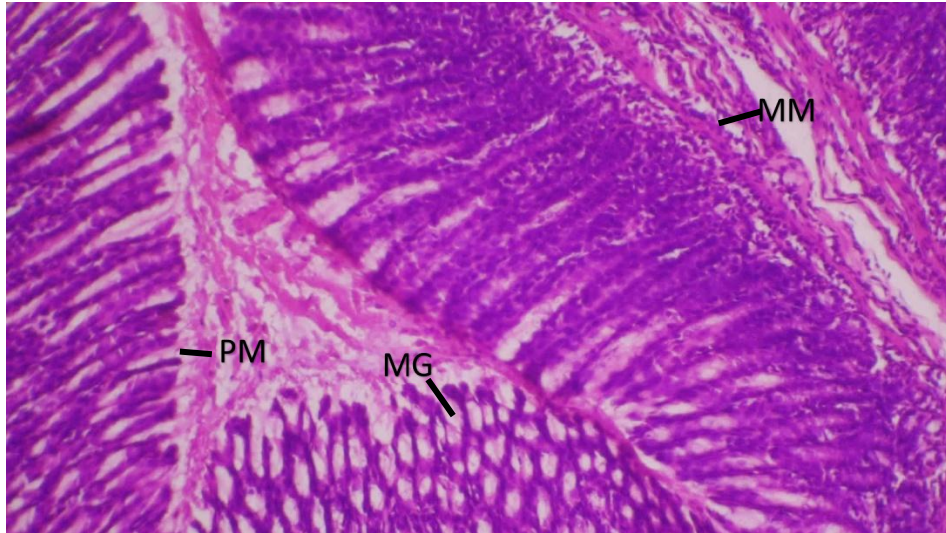


Plate 11: Rat stomach given Arsenic + low dose extract show: normal architecture: pitting mucosal lining (PM), mucosal glands (MG) and muscularis mucosa (MM) : H&E 100 X

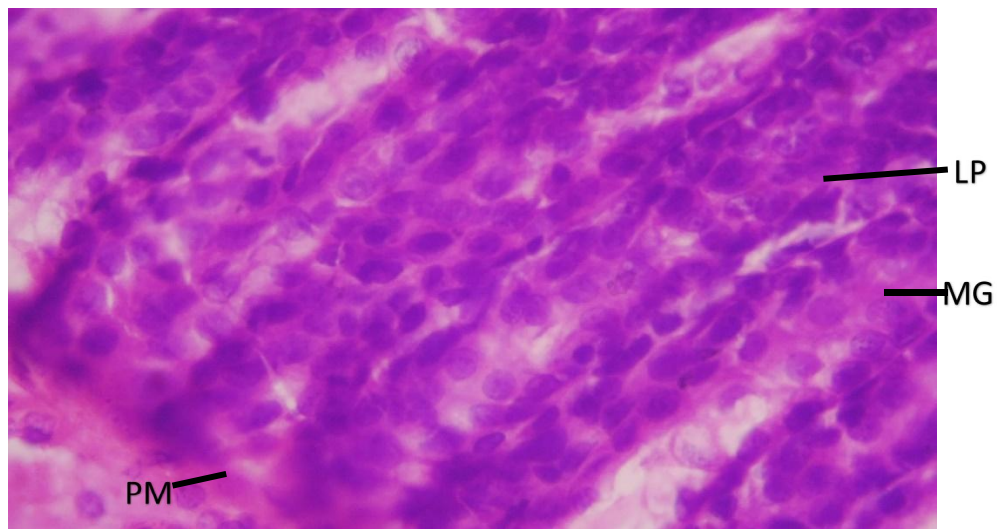


Plate 12: Rat stomach given Arsenic + low dose extract show: normal architecture: pitting mucosal lining (PM), mucosal glands (MG) and lamina propria (LP) : H&E 400 X

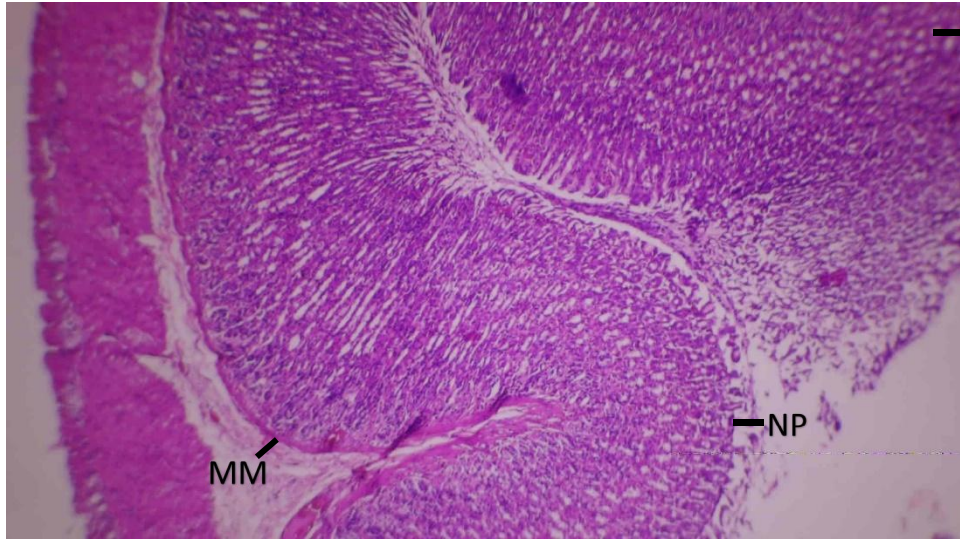


Plate 13. Rat stomach given Arsenic + high dose extract show: normal pitting mucosal lining (NP), mucosal glands (MG) and muscularis mucosa (MM): H&E 40 X

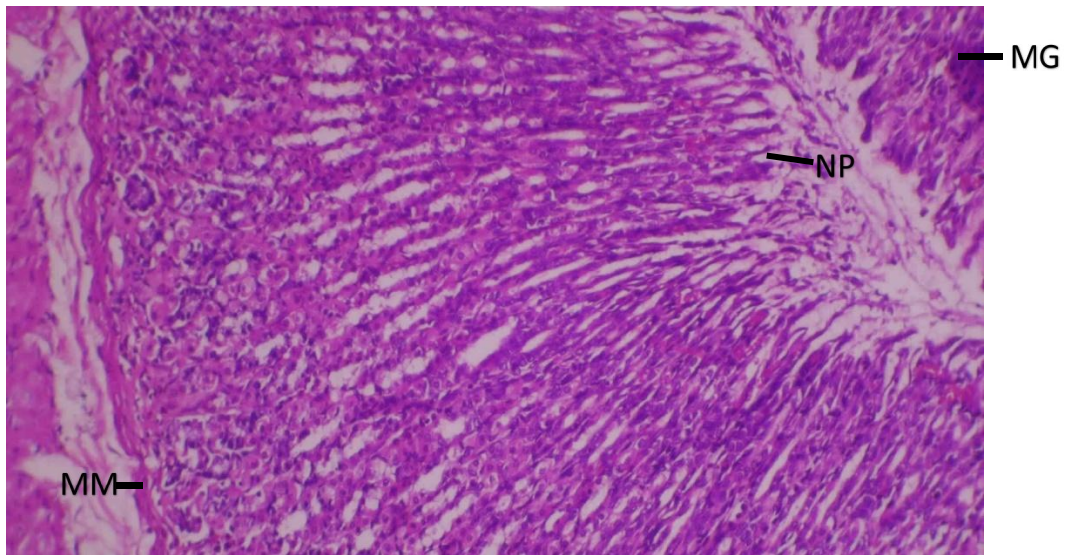


Plate 14. Rat stomach given Arsenic + high dose extract show: normal pitting mucosal lining (NP), mucosal glands (MG) and muscularis mucosa (MM): H&E 100 X

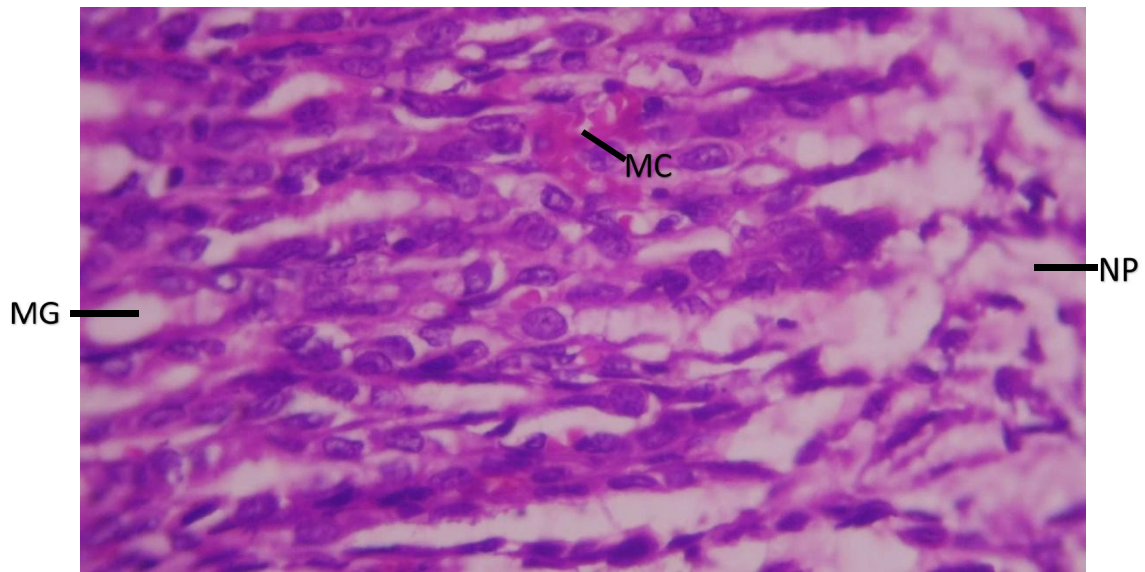


Plate 15. Rat stomach given Arsenic + high dose extract show: normal pitting mucosal lining (NP), mucosal glands (MG) and active mucosal congestion: H&E 400 X

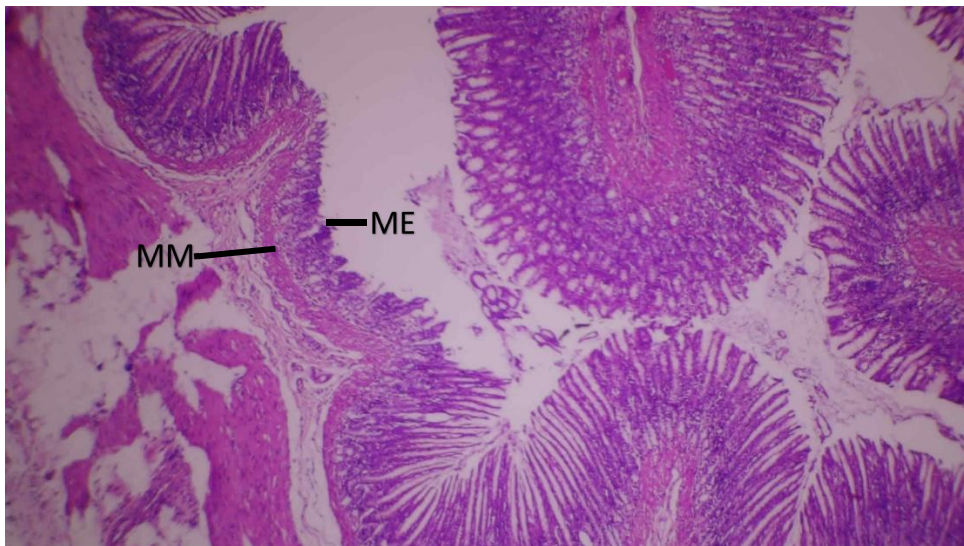


Plate 16. Rat stomach given Arsenic only (Reversal) show: severe mucosal erosion (ME), muscularis mucosa (MM): H&E 40 X

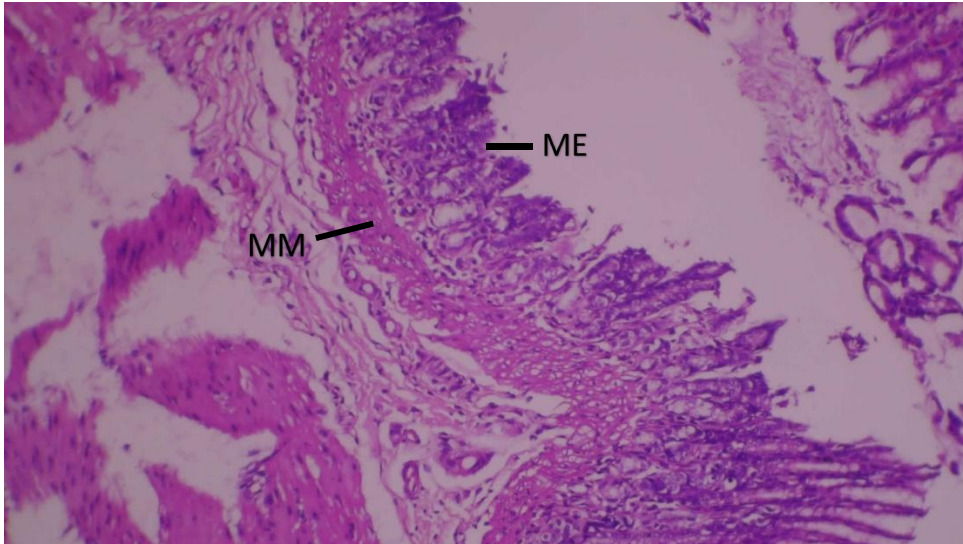


Plate 17. Rat stomach given Arsenic only (Reversal) show: severe mucosal erosion (ME), muscularis mucosa (MM) : H&E 100 X

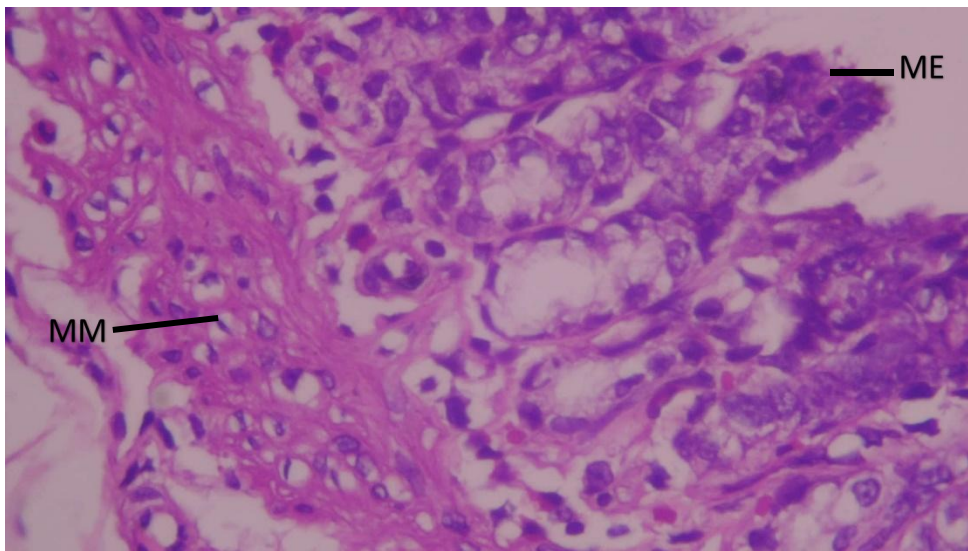


Plate 18. Rat stomach given Arsenic only (Reversal) show: severe mucosal erosion (ME), muscularis mucosa (MM) : H&E: 400 X

## CHAPTER FIVE

### 5.1. Discussion

The results findings of this study showed that the rats treated with arsenic trioxide only had a significant decrease in body weight compared to control group. This observation aligns with the findings of Imafidon & uwah , ( 2024), who also reported a significant reduction in rats exposed to arsenic trioxide. These consistent results suggest that arsenic trioxide exposure induces weight loss in rats, likely due to its toxic effects on metabolism and organ function. While the rats treated with arsenic trioxide followed by 250mg/kg body weight of camellia sinensis had significant increase in body weight compared to the arsenic trioxide only group. This observation contrasts with the findings of Chandra *et al.* (2011), who reported that the administration of camellia sinensis causes no significant change in the body weight. Our results might suggest a dose and context dependent effects of camellia sinensis: under a toxic insult (arsenic) where the plant extract might help restore weight loss. The rats treated with arsenic trioxide only had a significant decrease in organ weight compared to the control group.. The rats treated with arsenic trioxide only followed by 500mg/kg body weight of omeprazole had a significant increase in organ weight compared to arsenic trioxide only (Group B). The rats that were administered arsenic trioxide followed by 250mg/kg body weight of camellia sinensis extract showed significant increase in the organ weight compared to the arsenic trioxide only (Group D).

The anti-oxidant results showed that the rats treated with arsenic trioxide (Group B) only had significant decrease in the superoxide dismutase, catalase and glutathione peroxidase level while the malondialdehyde level was significantly increased compared to control. This observation aligns with the findings of Irshad *et al.* (2021), who reported that administration of arsenic significant reduced the SOD, CAT and GLP level and increased the MAD level in the rats. This suggest that arsenic exposure reduces anti-oxidant enzymes likely through lipid

peroxidation. In the rats treated with arsenic trioxide followed by 500mg/kg body weight of omeprazole there was a significant increase in the superoxide dismutase, catalase and glutathione peroxidase level while there was a significant decrease in the malondialdehyde level compared to arsenic trioxide only (Group B). In the rats treated with 250mg/kg body weight of camellia sinensis extract showed significant increase in the superoxide dismutase, catalase and glutathione peroxidase level while there was a significant decrease in the malondialdehyde level compared to arsenic trioxide only (Group B). In the rats treated with 500mg/kg body weight camellia sinensis extract showed significant increase in the superoxide dismutase, catalase and glutathione peroxidase level while there was a significant decrease in the malondialdehyde level compared to arsenic trioxide only (Group B). This observation aligns with the research finding of Uba *et al.* (2023), who reported that camellia sinensis extract elevated the SOD, CAT and GLTP level and reduced the MAD level in rats exposed to petrol fume induced oxidative stress. The difference in toxicant and dosing regimen in our study suggest that camellia sinensis may enhance anti oxidant enzyme in a dose and context dependent manner. The rats treated with arsenic trioxide only and left to recover had significant decrease in the superoxide dismutase, catalase and glutathione peroxidase level while the malondialdehyde level was significantly increased.

The histological analysis of the stomach sections revealed significant changes in the rats treated with arsenic. The rats treated with arsenic only showed severe crater ulceration and devitalized mucosa. While the rats treated with arsenic followed by graded doses of green tea extract showed significant improvements. The rats given the low dose of 250mg/kg body weight and high dose of 500mg/kg body weight showed completely resolved gastric ulcer.

However, the rats treated with arsenic followed by the standard drug (Omeprazole) showed partially resolved gastric ulcer. While, the rats treated with arsenic only and left to recover showed severe mucosa erosion and devitalized mucosa. Overall, the results demonstrate that

green tea extract at a dose of 250mg/kg body weight has a better ameliorative effect against arsenic-induced gastric damage in adult Wistar rats.

In conclusion, the results of this study demonstrate that aqueous leaf extract of *camellia sinensis* has protective effect against arsenic-induced gastric injury in adult Wistar rats. The extract's anti-inflammatory and antioxidant activity would probably account for this protective effect. The optimal dosage of the extract required to achieve this protective effect was found to be 250mg/kg body weight. These findings suggest that aqueous leaf extract of *camellia sinensis* could be a useful adjuvant treatment in preventing arsenic exposure-caused adverse effects on the stomach.

## **5.2. Conclusion**

This study investigated the effects of aqueous leaf extract of *camellia sinensis* on arsenic trioxide induced gastric damage in adult wistar rats. The findings of this research showed that the administration of arsenic trioxide can cause severe injury to the gastric mucosa, resulting in mucosa erosion, ulceration and oxidative stress in the mucosa. But treatment with aqueous leaf extract of *camellia sinensis* was found to greatly ameliorate these effects, decreasing inflammation, ulceration and oxidative stress in the lining. The most important findings of this study show that arsenic exposure resulted in ulceration and mucosal erosion. Treatment with aqueous leaf extract of *camellia sinensis* cured mucosa erosion, ulceration and decreased oxidative stress. The anti-inflammatory and anti-oxidant activities would probably account for the protective effects of the aqueous leaf extract of *camellia sinensis* on arsenic trioxide gastric damage. The results indicates that *camellia sinensis* has a better ameliorative effects compared to the standard anti-ulcer drug (omeprazole) administered.



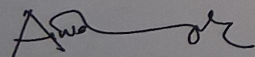
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## APPENDIX

	<b>RESEARCH ETHICS COMMITTEE</b> COLLEGE OF MEDICAL SCIENCES UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.	
<b>Chairman:</b> Prof. F. A Imarhiagbe MBChb, FMCP Cert Clin Res and ethics (NIH), MD. 0803449092	<b>Email:</b> researchethics.cms@gmail.com	P.M.B 1154, BENIN CITY
<b>Our Ref:</b> CMS/REC/01/VOL.2/780	<b>Date:</b> 18 <sup>th</sup> September, 2025	
<b>Re: INVESTIGATING THE EFFECT OF AQUEOUS LEAF EXTRACT OF CAMELLIA SINENSIS ON ARSEN INDUCES GASTRIC DAMAGE IN ADULT WISTAR RATS</b>		
<b>Name of Principal Investigator:</b>	<b>ABIOLA OYINOLUWA MERCY</b> Department Of Anatomy, School of Basic Medical Science College of Medical Sciences, University of Benin	
<b>REC Approval No:</b>	CMS/REC/2025/780	
This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.		
This approval dates from 18 <sup>th</sup> September, 2025 to 19 <sup>th</sup> September, 2026. In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.		
The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice. Thank you.		
		
<b>PROF. F.A IMARHIAGBE</b> Chairman, REC		
<i>Promoting best ethical &amp; scientific standard for research in Nigeria</i>		