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

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UNIVERSITY OF BENIN

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

IN PARTIAL FULFILMENT OF THE

REQUIREMENT FOR THE AWARD OF

BACHELOR OF SCIENCE (B.s.c) INANATOMY.

UNIVERSITY OF BENIN

BENIN CITY, EDO STATE

NIGERIA.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research was carried out by **ABIODE FRANCESS OSHIOKUWE** matriculation number **BMS2101297** in the department of anatomy school of basic medical sciences, university of Benin, Benin city, Nigeria. In partial fulfilment for the award of bachelor of science (B.Sc) degree.

_____ _____

DR IMAFIDON **DATE**

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DR ADAZE.B. ENOGIERU **DATE**

(HEAD OF DEPARTMENT)

_____ _____

(EXTERNAL EXAMINER) **DATE**

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DEDICATION

I dedicate this project to God Almighty for his Grace and favour throughout my undergraduate studies and to my parents Mr. and Mrs. Abiode, my siblings, my Aunty, my uncle and everyone who contributed to the 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. Abiode), my lovely aunty, Mrs. Bridget Aigbokhai and my siblings (Monica, Kenneth and Celestine) 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.

I would also like to acknowledge the contributions of my coursemate, Abiola Oyinoluwa for her collaborations in bringing this project work to a success. I appreciate my friends, Abu Dorcas, Oviosun Fulness, Mudiaga-John Precious, Akinseinde Eunice for the unwavering love and support through the process. I specially thank Omozuafo Isaac and Mr. Samuel Nwamgbada for their immerse support and assistance during the course of this project.

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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Fig 4.7**:** showing Malondialdehyde concentration across the experimental groups. Values are given as Mean ±SEM. * $p < 0.05$ compared with control group; # $p < 0.05$ compared with Arsenic only group.

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ABSTRACT

Green tea (*camellia sinensis)* is rich in bioactive compounds, particularly catechins like epigallocatechin gallate (EGCG), epicatechin and flavonoids. Thes constituents contribute to its well-documented antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic properties. Arsenic contamination represents a serious environmental and health hazard, particularly in developing nations where regulatory oversight of water and food quality is often lacking. Among the various organs affected, the gastrointestinal tract, especially the **duodenum,** is a primary site of arsenic-induced toxicity due to its direct contact with ingested substances. One of the primary mechanisms by which arsenic exerts its toxic effects is through the generation of **oxidative stress,** which leads to cellular injury, inflammation, and apoptosis. This study investigated the protective effects of aqueous leaf extract of *camellia sinensis* on arsenic-induced duodenal damage in adult Wistar rats. The study used adult Wistar rats that were randomly divided into six groups (A-F). The first group A(control) were given feed and water, while the rats in group B were administered arsenic trioxide 10mg/kg body weight for 14days and sacrificed by cervical dislocation afterwards. The rats in group C were administered arsenic trioxide 10mg/kg body weight for 14 days and standard drugs(omeprazole) for 28 days. The rats in group D were administered arsenic trioxide 10mg/kg body weight for 14 days and aqueous leaf extract of *camellia sinensis* 250mg/kg body weight for 28 days while group E were administered arsenic trioxide 10mg/kg body weight for 14 days and aqueous leaf extract of *camellia sinensis* 500mg/kg body weight for 28days. The rats in group F were administered arsenic trioxide 10mg/kg for 14days and allowed to recover for 28days. The results showed that the rats in group A (control) show normal tissue architecture with well-defined mucosal lining, crypts, lamina propria, muscularis, mucosa and submucosa. However, the rats treated with arsenic only (group B) show severe atrophy, mural infiltrates of inflammatory cells and cell congestion, similar findings were observed in group C, rats treated with omeprazole (mild degree) and the group F treated with only arsenic and allowed to recover showed comparable degrees. Also, treatment with aqueous leaf extract of *camellia sinensis* at a dose of 250mg/kg (group D), completely resolved chronic duodenitis, while high dose of 500mg/kg body weight (group E), mildly resolved chronic duodenitis. Findings also showed that there were increased oxidative stress markers in rats treated with arsenic trioxide only and similar findings was also seen in the group treated with omeprazole and the group treated with arsenic and allowed to recover. The first group(control) has great level of anti-oxidant enzymes, similar findings were observed in the groups treated with aqueous leaf extract of *camellia sinensis*. The findings in the study suggest that aqueous leaf extract of *camellia sinensis* has a protective effects against arsenic-induced duodenal injury in adult Wistar rats.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Arsenic contamination represents a serious environmental and health hazard, particularly in developing nations where regulatory oversight of water and food quality is often lacking. Chronic exposure to arsenic, commonly through contaminated drinking water or food, has been associated with numerous health complications, including gastrointestinal damage, cancer, cardiovascular diseases, and neurological disorders (Naujokas et al., 2013). Among the various organs affected, the gastrointestinal tract, especially the duodenum, is a primary site of arsenic-induced toxicity due to its direct contact with ingested substances (Mazumder, 2008).

One of the primary mechanisms by which arsenic exerts its toxic effects is through the generation of oxidative stress, which leads to cellular injury, inflammation, and apoptosis. Arsenic increases the production of reactive oxygen species (ROS), disrupts antioxidant defenses, and activates inflammatory pathways in intestinal tissues (Flora, 2011). Prolonged oxidative stress in the gastrointestinal mucosa may lead to mucosal damage, ulceration, and impaired nutrient absorption (Jomova et al., 2011).

Recent attention has turned to the use of natural antioxidants as protective agents against heavy metal toxicity. Green tea (*Camellia sinensis*), a widely consumed beverage, has shown promising antioxidant, anti-inflammatory, and metal-chelating properties attributed mainly to its polyphenolic compounds, especially catechins like epigallocatechin gallate (EGCG) (Cabrera et al., 2006). These compounds are known to scavenge free radicals, upregulate endogenous antioxidant enzymes, and modulate inflammatory signaling pathways (Lambert et al., 2005). Previous studies have demonstrated the efficacy of green

tea in mitigating oxidative damage in various tissues, including the liver, kidney, and brain (Khan & Mukhtar, 2007), but limited research has been conducted on its protective effect against arsenic-induced duodenal damage.

Given the prevalence of arsenic exposure and the limited therapeutic options available, there is a compelling need to investigate natural, accessible, and cost-effective interventions. This study, therefore, aims to evaluate the protective role of aqueous leaf extract of *Camellia sinensis* against arsenic trioxide-induced duodenal damage in adult Wistar rats. The findings may provide insights into the development of nutraceutical interventions for populations at risk of arsenic exposure and validate the traditional use of green tea in gastrointestinal health management.

1.2 JUSTIFICATION OF THE STUDY

This study is important because it explores a natural, widely available and cost-effective intervention of arsenic induced gastrointestinal toxicity. *Camellia sinensis* (Green tea) extract is known for its antioxidant and anti-inflammatory properties, but its role in protecting against duodenal damage from arsenic toxicity is not well-documented. Findings from this study could contribute to the development of dietary recommendations or therapeutic interventions for populations exposed to arsenic –contaminated environments. The study may provide scientific validation for traditional medicinal uses of *camellia sinensis* in gastrointestinal protection. Arsenic trioxide-induced duodenum damage is a significant public concern, with chronic exposure leading to inflammation, ulceration and potentially even duodenal ulcer.

1.3. STATEMENT OF PROBLEM

Arsenic contamination remains a significant environmental and health issue, particularly in developing countries where water and food safety regulations are not strictly enforced.

Despite increasing research on antioxidant-based interventions, limited studies have explored the protective effects of green tea extract specifically on duodenal damage caused by arsenic toxicity. Investigating the mechanisms by which *camellia sinensis* alleviates oxidative stress and inflammation in the duodenum could contribute to the development of natural and affordable treatment strategies for arsenic toxicity.

1.4. SIGNIFICANCE OF THE STUDY

This research will contribute to scientific knowledge on the protective effects of green tea and therapeutic insights for arsenic-induced duodenal damage, review the medicinal properties of *camellia sinensis* and offer data that may guide future pharmacological and natural health product developments.

1.5. AIM OF STUDY

The aim of this study is to investigate the effects of aqueous leaf extract of *Camellia sinensis* on arsenic trioxide toxin-induced duodenal damage in adult Wistar rat.

1.6. OBJECTIVES OF THE STUDY

- To evaluate the effect of aqueous leaf extract of *Camellia sinensis* on the body weight changes in wistar rats induced with arsenic trioxide
- To evaluate the biochemical effects of aqueous *Camellia sinensis* extract on oxidative stress markers in arsenic trioxide exposed Wistar rats.
- To assess the histopathological changes in the duodenum following arsenic trioxide exposure and green tea treatment.

1.7. RESEARCH QUESTIONS

- Does aqueous *camellia sinensis* extract reduce oxidative stress markers in arsenic-induced duodenal toxicity?
- How does *Camellia sinensis* influence the expression of inflammatory and antioxidant-related genes in the duodenum?

- What are the histopathological effects of arsenic exposure on the duodenum, and can *camellia sinensis* mitigate these effects?

1.8. 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 duodenal 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

2.1.1. PHYSICAL PROPERTIES

Arsenic trioxide (As_2O_3) is a white, crystalline solid that is odorless and tasteless. It has a melting point of 312°C and sublimes at high temperatures. It is poorly soluble in cold water but more soluble in hot water, forming a slightly acidic solution due to the formation of arsenous acid (H_3AsO_3). These physical characteristics facilitate its bioavailability in environmental settings, allowing it to persist in water sources and soil (ATSDR, 2007).

2.1.2. CHEMICAL PROPERTIES

Chemically, arsenic trioxide consists of arsenic in the trivalent oxidation state. This trivalent form has a high affinity for thiol (-SH) groups in proteins, leading to the inhibition of enzyme activity. It can readily oxidize and undergo redox reactions that disturb the cellular oxidative balance (Hughes, 2002). Its affinity for sulfhydryl groups makes it particularly toxic to proteins and enzymes essential for cell survival.

2.1.3. SOURCES AND EXPOSURE ROUTES

Arsenic trioxide is both naturally occurring and anthropogenic. Natural sources include volcanic activity, mineral erosion, and groundwater leaching from arsenic-containing rocks. Anthropogenic sources include mining, smelting, herbicide and pesticide usage, and pharmaceuticals, notably in the treatment of acute promyelocytic leukemia (WHO, 2011). Exposure can occur through contaminated drinking water, occupational inhalation, ingestion of food grown in contaminated soil, and dermal contact (Jomova et al., 2011).

2.1.4. MECHANISM OF TOXICITY

Arsenic trioxide exerts its toxic effects primarily through oxidative stress mechanisms. It promotes the generation of reactive oxygen species (ROS), leading to lipid peroxidation, DNA damage, and protein oxidation (Bisht & Nehru, 2005). It disrupts mitochondrial function, inhibits oxidative phosphorylation, and induces apoptosis through caspase activation. Arsenic also interferes with DNA repair systems and cellular signaling pathways, contributing to genotoxic and carcinogenic effects (Singh et al., 2014).

2.1.5. EFFECTS ON THE GASTROINTESTINAL TRACT

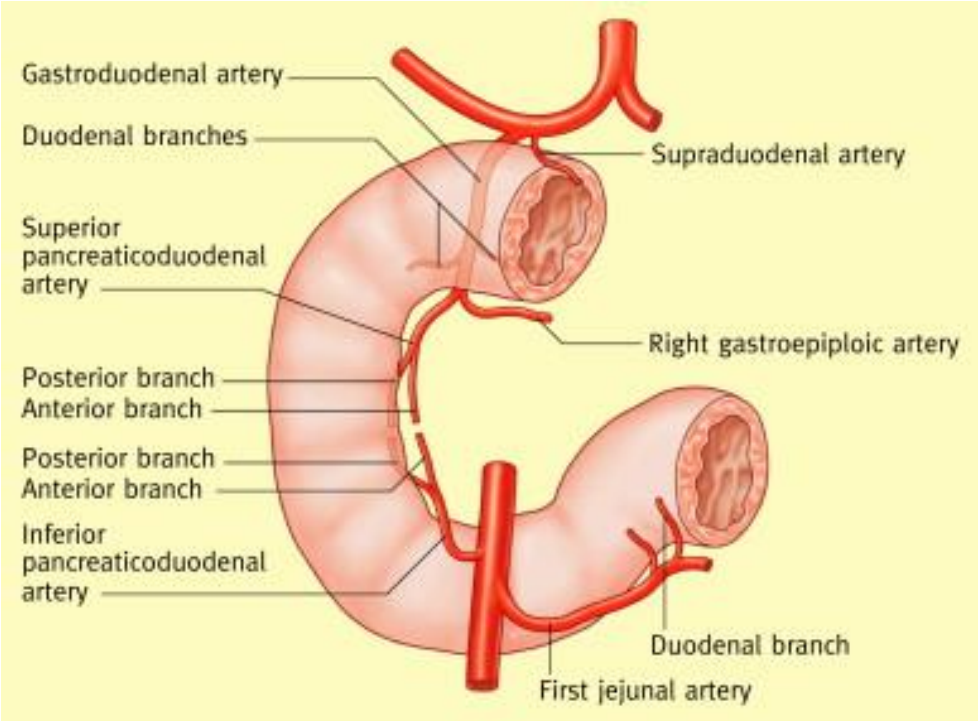
Chronic exposure to arsenic trioxide adversely affects the gastrointestinal tract, particularly the duodenum. Clinical and animal studies have shown that arsenic disrupts

intestinal epithelial integrity, reduces villus height, induces crypt hyperplasia, and causes mucosal erosion. These changes impair nutrient absorption and increase intestinal permeability, contributing to gastrointestinal symptoms such as diarrhea, nausea, and abdominal pain (Jomova et al., 2011; Singh et al., 2014).

2.1.6. COMMON HISTOLOGICAL FINDINGS

Histological studies of arsenic-exposed duodenum commonly show villus atrophy, crypt elongation, and epithelial degeneration. There is often marked infiltration of inflammatory cells and degeneration of Brunner's glands. The mucosal damage compromises the absorptive function of the duodenum, increasing the risk of systemic exposure to other toxins and pathogens (Ali et al., 2015).

2.2.THE DUODENUM



Heller, M.,. (2013). Duodenum: *Clinical Radiology*, *69*(1), e48–e55. https://doi.org/10.1016/j.crad.2013.09.013

2.2.1. LOCATION AND POSITION

The duodenum is the first segment of the small intestine, extending from the pylorus of the stomach to the jejunum. It lies retroperitoneally and forms a C-shaped curve around the head of the pancreas (Heller, 2013). It is approximately 25–30 cm long and situated opposite the first and second lumbar vertebrae.

2.2.2. SHAPE AND SIZE

The duodenum is roughly C-shaped, comprising four parts; the superior, descending, horizontal, and ascending segments. Its diameter is about 2.5–3 cm, making it slightly wider than other parts of the small intestine (Guyton & Hall, 2011).

2.2.3. PARTS OF THE DUODENUM

Superior (First) Part: Extends from the pylorus and is intraperitoneal for its proximal 2 cm.

Descending (Second) Part: Receives the openings of the common bile duct and pancreatic duct.

Horizontal (Third) Part: Crosses the midline anterior to the aorta and inferior vena cava.

Ascending (Fourth) Part: Ascends to the duodenojejunal flexure supported by the ligament of Treitz (Sadler, 2012).

2.2.4. BLOOD SUPPLY

The duodenum receives arterial blood from the superior pancreaticoduodenal artery (a branch of the gastroduodenal artery) and the inferior pancreaticoduodenal artery (a branch of the superior mesenteric artery). Venous drainage is via the pancreaticoduodenal veins, which empty into the portal vein (Junqueira & Carneiro, 2005).

2.2.5. INNERVATION

The duodenum receives parasympathetic innervation from the vagus nerve and sympathetic fibers from the celiac plexus. These regulate motility, secretion, and vascular tone (Guyton & Hall, 2011).

2.2.6. CLINICAL SIGNIFICANCE

Due to its anatomical proximity to the pancreas and biliary system, the duodenum is often affected by diseases such as duodenal ulcers, duodenitis, and obstructive lesions. It also serves as a key diagnostic site for biopsy in malabsorption syndromes and celiac disease (Greenberg & Podolsky, 2020)

2.2.7. HISTOLOGY OF THE DUODENUM

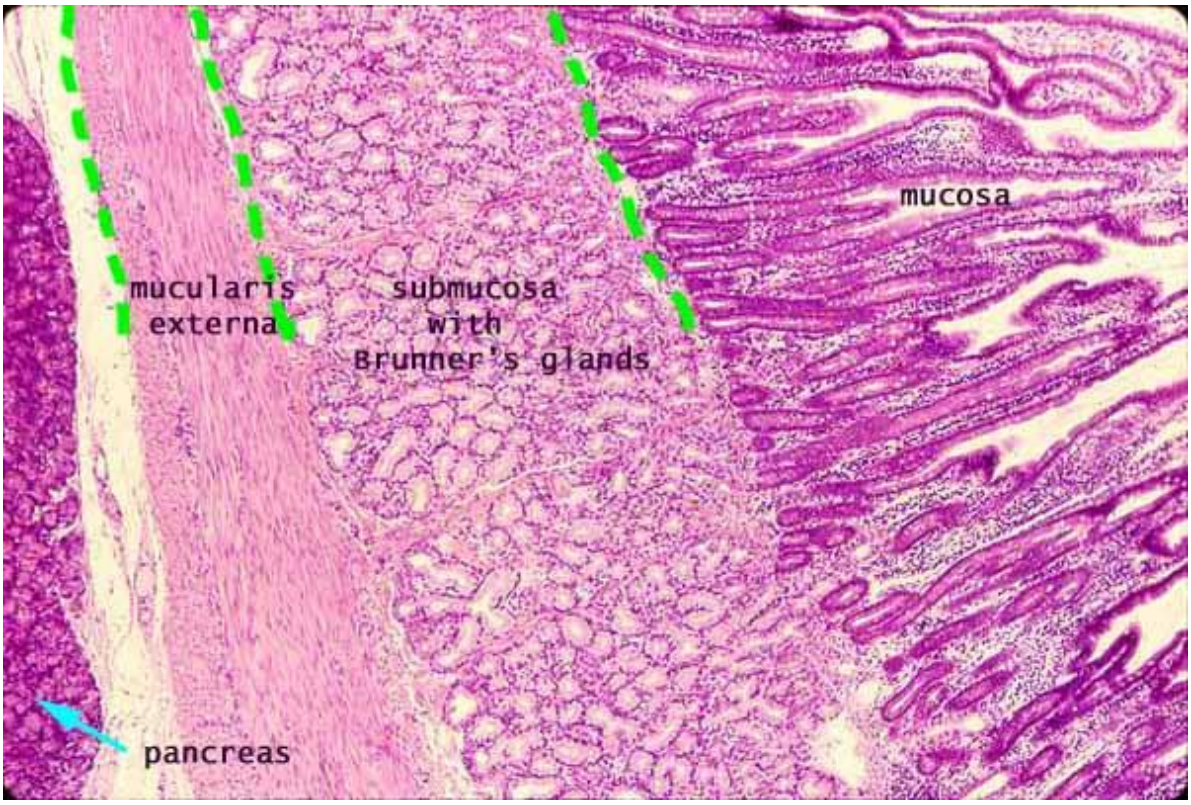


Figure 2.2: Histological Layers of the Duodenum

2.2.8 LAYERS OF THE DUODENUM

Mucosa: Contains villi and crypts of Lieberkühn. The villi increase the surface area for absorption, while the crypts secrete digestive enzymes.

Submucosa: Contains Brunner's glands, which secrete alkaline mucus to neutralize gastric acid.

Muscularis Externa: Composed of inner circular and outer longitudinal muscle layers that facilitate peristalsis.

Serosa (Adventitia): A thin connective tissue layer that anchors the duodenum to surrounding structures (Junqueira & Carneiro, 2005).

2.2.9. CELLS TYPES FOUND IN EACH LAYER

Enterocytes: Absorptive epithelial cells lining the villi.

Goblet Cells: Secrete mucus for lubrication and protection.

Enteroendocrine Cells: Release hormones such as secretin and cholecystokinin.

Paneth Cells: Found at the base of crypts; secrete antimicrobial enzymes.

Stem Cells: Responsible for continuous renewal of the intestinal epithelium.

2.2.10. CLINICAL SIGNIFICANCE

The duodenum plays a central role in various gastrointestinal disorders due to its anatomical position, functional importance, and close relationship with surrounding organs. Common clinical conditions affecting the duodenum include duodenal ulcers, often caused by *Helicobacter pylori* infection or prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), which disrupt mucosal defenses and lead to erosion of the mucosal lining (Sung et al., 2009). Duodenitis, an inflammation of the duodenal mucosa, may occur secondary to infections, celiac disease, or exposure to irritants. The

duodenum can also be affected by obstructive lesions such as tumors, strictures, or external compression from pancreatic or biliary pathology, resulting in symptoms like vomiting, abdominal pain, and malabsorption (Greenberg & Podolsky, 2020).

Additionally, because the duodenum is the primary site for iron, calcium, and magnesium absorption, diseases that damage its mucosa, such as celiac disease, can lead to significant micronutrient deficiencies (Ferri, 2022). Duodenal injury or resection can impair digestion and nutrient assimilation, while congenital anomalies like duodenal atresia and annular pancreas present early in life with obstruction. Clinically, duodenal biopsies are frequently used in diagnosing malabsorptive disorders, small intestinal bacterial overgrowth, and inflammatory conditions, underscoring the duodenum's diagnostic and therapeutic importance.

2.2.11. DEVELOPMENT

During embryonic development, the duodenum arises from the distal foregut and proximal midgut. Its dual origin is reflected in its dual blood supply and innervation. Rotation and fusion with the pancreas during development result in most of the duodenum becoming secondarily retroperitoneal, except for the proximal portion (Sadler, 2012).

2.2.12. FUNCTIONS OF THE DUODENUM

The duodenum plays a vital role in digestion and regulation of gastrointestinal. Its major functions include:

1. **Neutralization of gastric acid** through alkaline mucus from Brunner's gland

2. **Regulation of gastric emptying** via hormonal feedback (secretin and cholecystokinin).
3. **Coordination of pancreatic enzyme and bile secretion.**
4. **Absorption of nutrients**, particularly iron, calcium, and magnesium.
5. **Regulation of intestinal pH** for optimal enzyme activity.
6. **`Facilitation of peristaltic movement** to mix chyme and secretions.

2.2.13. EVALUATION CRITERIA IN WISTAR RATS

Experimental studies involving Wistar rats use several criteria to evaluate duodenal integrity, including villus height, crypt depth, 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. CAMELLIA SINENSIS (GREEN TEA)

Green Tea: Usefulness and Safety. NCCIH. Published 2020.

https://www.nccih.nih.gov/health/green-tea

2.3.1 SCIENTIFIC CLASSIFICATION

Taxonomic Rank	Classification
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida

Taxonomic Rank	Classification
Order	Ericales
Family	Theaceae
Genus	*Camellia*
Species	*Camellia sinensis*

2.3.2 PHYTOCHEMICAL PROPERTIES

- Major phytochemical constituents of *Camellia sinensis* include:
- **Catechins (e.g., EGCG, EGC):** Potent antioxidants that neutralize ROS.
- **Flavonoids:** Exhibit anti-inflammatory and vasoprotective activities
- **Caffeine:** Stimulates metabolism and enhances alertness.
- **Theanine:** Promotes relaxation and neuroprotection.
- **Tannins:** Possess antimicrobial and astringent effects.
- **Saponins:** Show immune-modulatory and cholesterol-lowering actions.

2.3.3. BIOLOGICAL EFFECTS

Numerous studies have shown that *Camellia sinensis* exhibits strong antioxidant activity by scavenging free radicals and enhancing the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. It also reduces inflammation by inhibiting pro-inflammatory cytokines and pathways such as NF-κB. These effects help protect tissues from oxidative and inflammatory damage (Lambert et al., 2005).

2.3.4. EVIDENCE FROM PRIOR STUDIES

Several animal studies have shown that green tea extracts can ameliorate arsenic-induced damage in various organs, including the gastrointestinal tract. Green tea has been reported to preserve villus height and crypt depth, reduce inflammatory cell infiltration, and enhance mucosal regeneration in rats exposed to toxicants (Katiyar et al., 2001). These studies support the potential of green tea as a therapeutic agent in arsenic-induced toxicity

2.3.5. MEDICINAL AND PHARMACEUTICAL EFFECTS

Green tea (*Camellia sinensis*) has been extensively studied for its diverse medicinal and pharmaceutical applications, attributed mainly to its high content of catechins, particularly epigallocatechin gallate (EGCG). Medicinally, green tea demonstrates cardioprotective effects by improving endothelial function, reducing low-density lipoprotein (LDL) oxidation, and modulating blood pressure (Kuriyama et al., 2006). Its antidiabetic potential is linked to improved insulin sensitivity and reduced postprandial glucose levels through modulation of carbohydrate-digesting enzymes and glucose transporters (Cabrera et al., 2006).

Pharmacologically, green tea exhibits neuroprotective properties, with studies indicating reduced risk of neurodegenerative diseases such as Alzheimer's and Parkinson's through suppression of oxidative damage, inhibition of β-amyloid aggregation, and modulation of dopaminergic pathways (Mandel et al., 2011). Its anticarcinogenic activity has been explored in chemoprevention, where polyphenols inhibit tumor

initiation and progression by modulating signal transduction pathways, inducing apoptosis, and inhibiting angiogenesis (Yang et al., 2009).

In pharmaceutical development, green tea polyphenols are being incorporated into drug delivery systems for enhanced bioavailability and synergistic therapeutic effects. Nanoencapsulation of EGCG, for example, improves its stability, absorption, and targeted delivery, increasing its therapeutic potential in managing chronic diseases (Khan & Mukhtar, 2013). Additionally, green tea extracts are used in topical formulations for dermatological benefits, such as photoprotection, anti-aging, and treatment of inflammatory skin conditions, due to their antioxidant and anti-inflammatory actions (Katiyar, 2011). These multifaceted effects make green tea an important candidate for both preventive and adjunctive therapeutic strategies.

2.3.6. MECHANISM OF PROTECTION

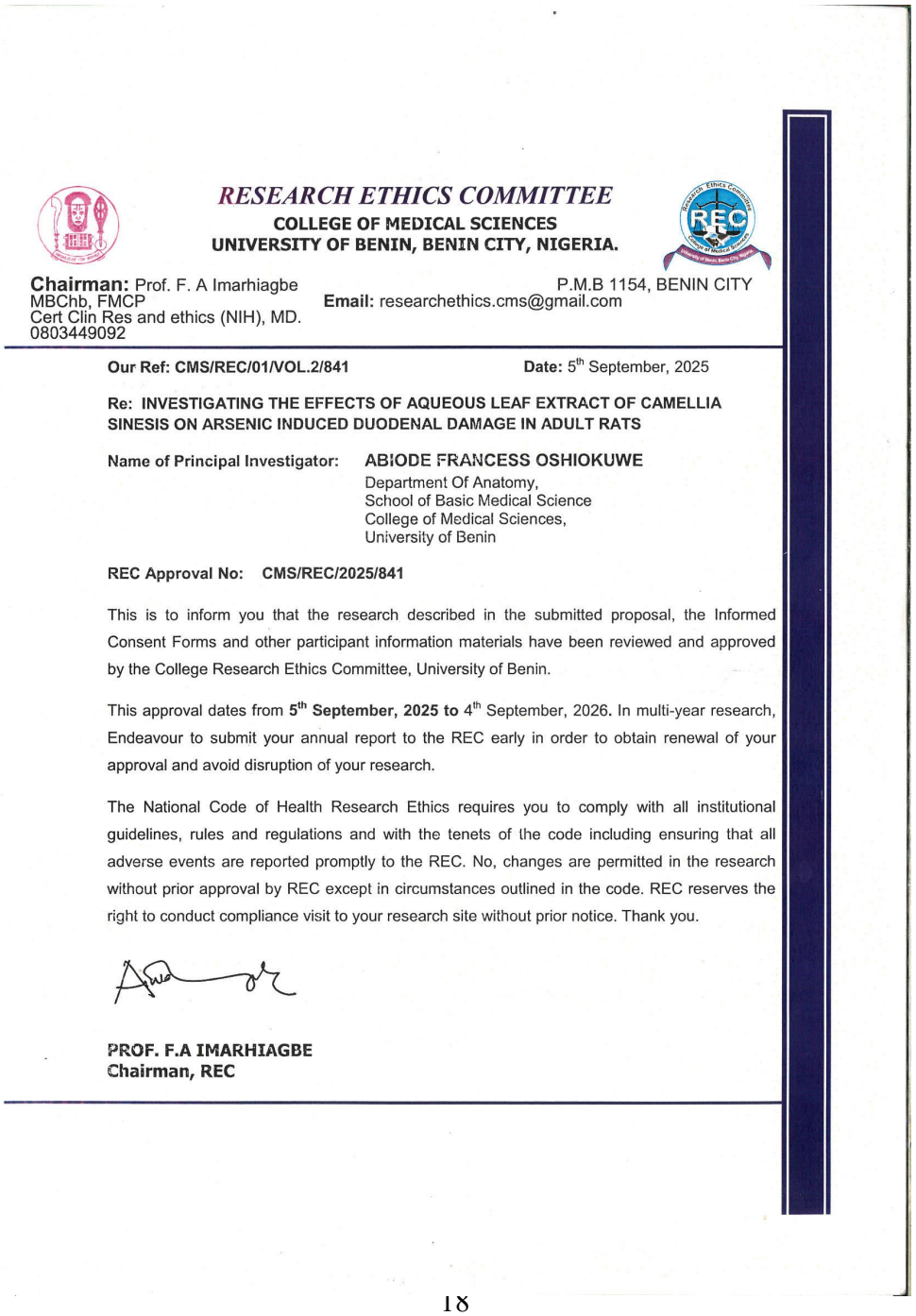
The protective mechanism of *Camellia sinensis* against arsenic toxicity involves multiple pathways. EGCG and other polyphenols act as metal chelators, reducing arsenic bioavailability. They inhibit oxidative stress by neutralizing ROS and upregulating cellular defense enzymes. Green tea also stabilizes mitochondrial function and promotes epithelial cell regeneration, enhancing overall tissue integrity (Lambert et al., 2005).

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/2025/841.



RESEARCH ETHICS COMMITTEE
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

Chairman: Prof. F. A Imarhiagbe
MBChb, FMCP
Cert Clin Res and ethics (NIH), MD.
0803449092

P.M.B 1154, BENIN CITY
Email: researchethics.cms@gmail.com

Our Ref: CMS/REC/01/VOL.2/841 **Date:** 5th September, 2025

Re: INVESTIGATING THE EFFECTS OF AQUEOUS LEAF EXTRACT OF CAMELLIA SINESIS ON ARSENIC INDUCED DUODENAL DAMAGE IN ADULT RATS

Name of Principal Investigator: **ABIODE FRANCESS OSHIOKUWE**
Department Of Anatomy,
School of Basic Medical Science
College of Medical Sciences,
University of Benin

REC Approval No: CMS/REC/2025/841

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 **5th September, 2025 to** 4th 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.

PROF. F.A IMARHIAGBE
Chairman, REC

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 PLANT MATERIAL

REAGENT 1: *Camellia sinensis leaf*

Processed *Camellia sinensis* leaf was gotten from the supermarket, Stop 2 shop supermarket, opposite the university of Benin,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, 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 42days.

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	42days
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 28days	42 days
Group E	10mg/kg body weight of Arsenic trioxide for 14days + 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 duodenum was removed and fixed in 1% formal saline for histological analysis.

3.9. HISTOLOGICAL PROCEDURE

Duodenual 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 duodenal 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 ± 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

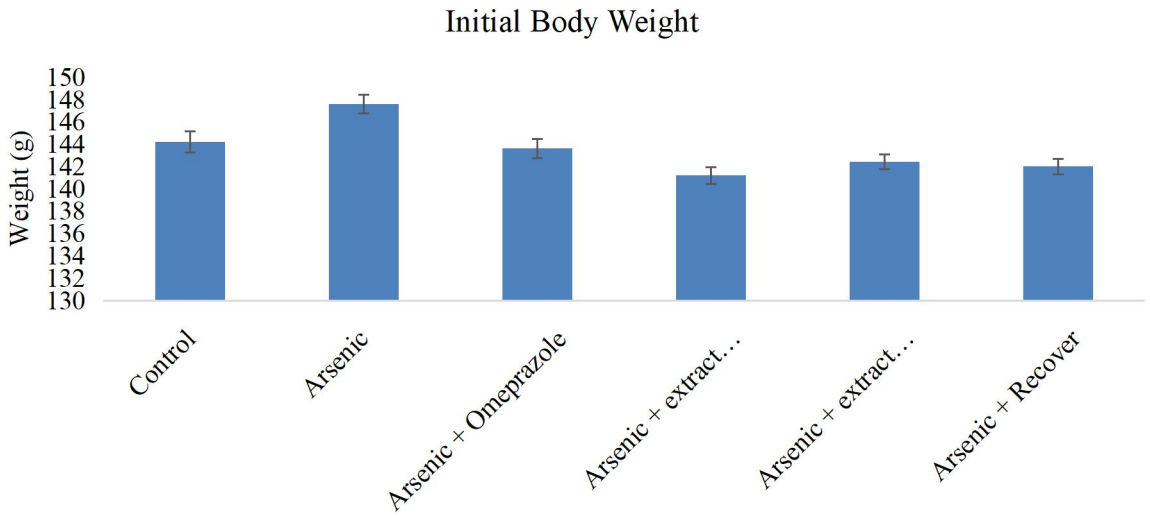


Figure 4.1: Chart showing initial body weight across the experimental groups. Values are given as Mean ±SEM. * p <0.05 compared with control group; # p <0.05 compared with Arsenic only group.

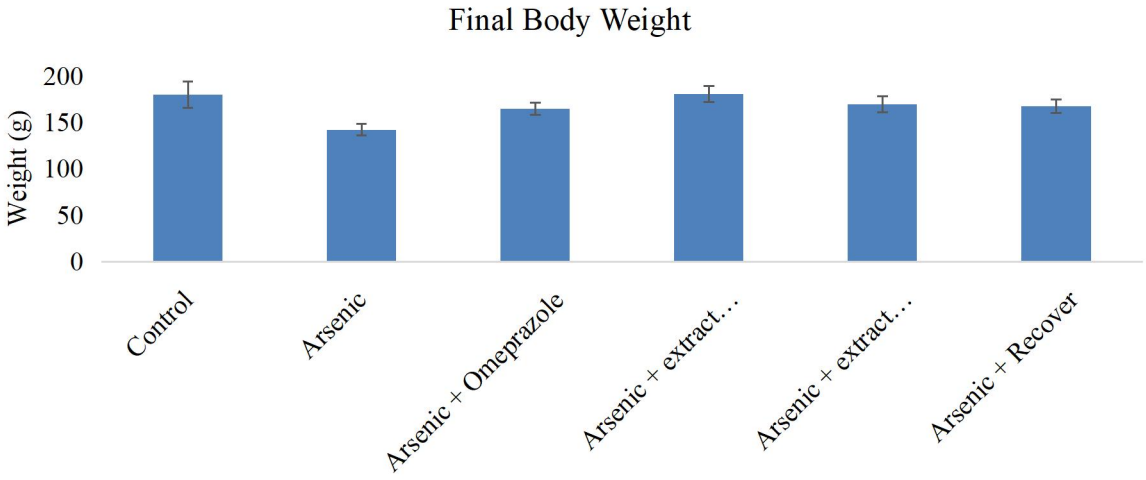


Figure 4.2: Chart showing final body weight across the experimental groups. Values are given as Mean ±SEM. * p <0.05 compared with control group; # p <0.05 compared with Arsenic only group

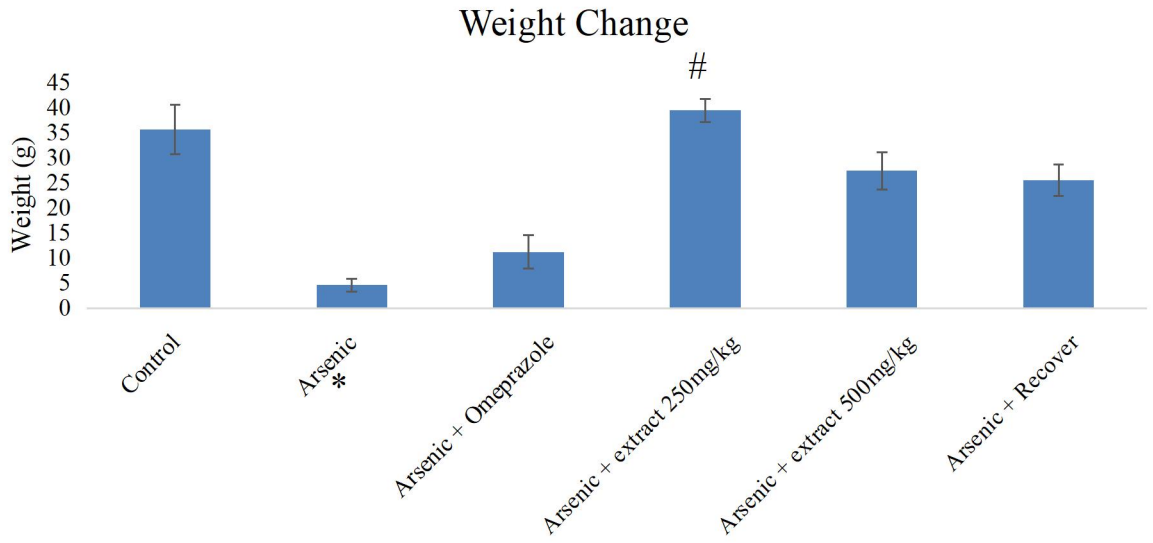


Figure 4.3: Chart showing body weight changes across the experimental groups. Values are given as Mean ±SEM. * p <0.05 compared with control group; # p <0.05 compared with Arsenic only group.

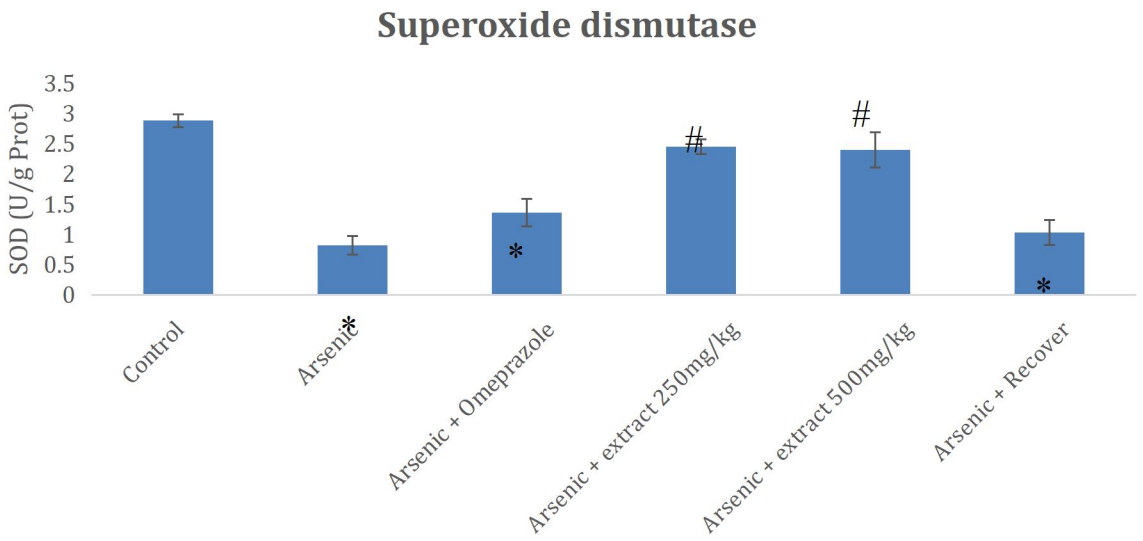


Figure 4.4: Chart showing superoxide dismutase activity across the experimental groups. Values are given as Mean ±SEM. * p <0.05 compared with control group; # p<0.05 compared with Arsenic only group.

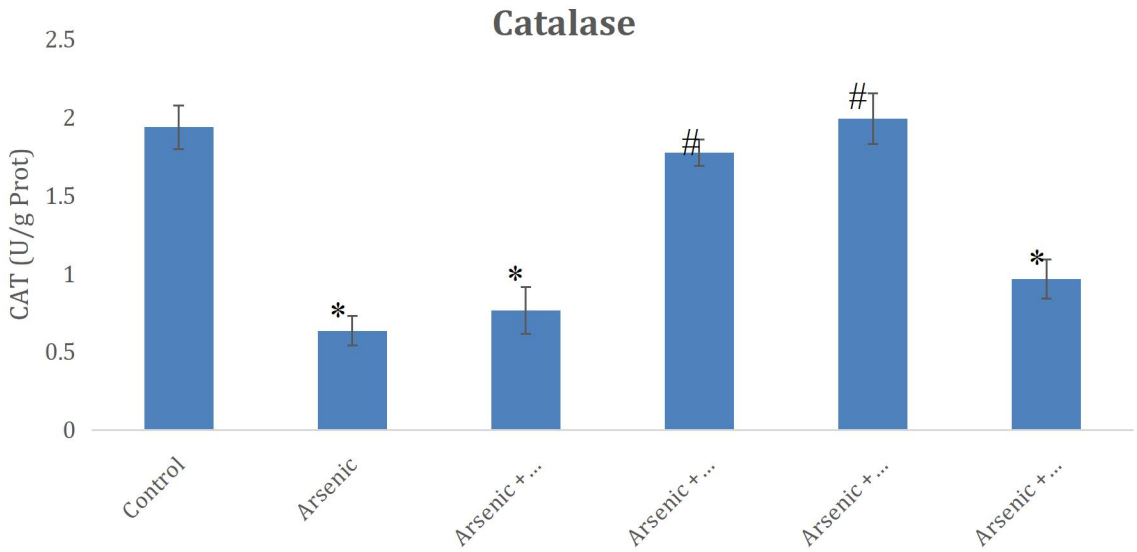


Figure 4.5: Chart showing Catalase activity across the experimental groups. Values are given as Mean ±SEM. * p <0.05 compared with control group; # p<0.05 compared with Arsenic only group.

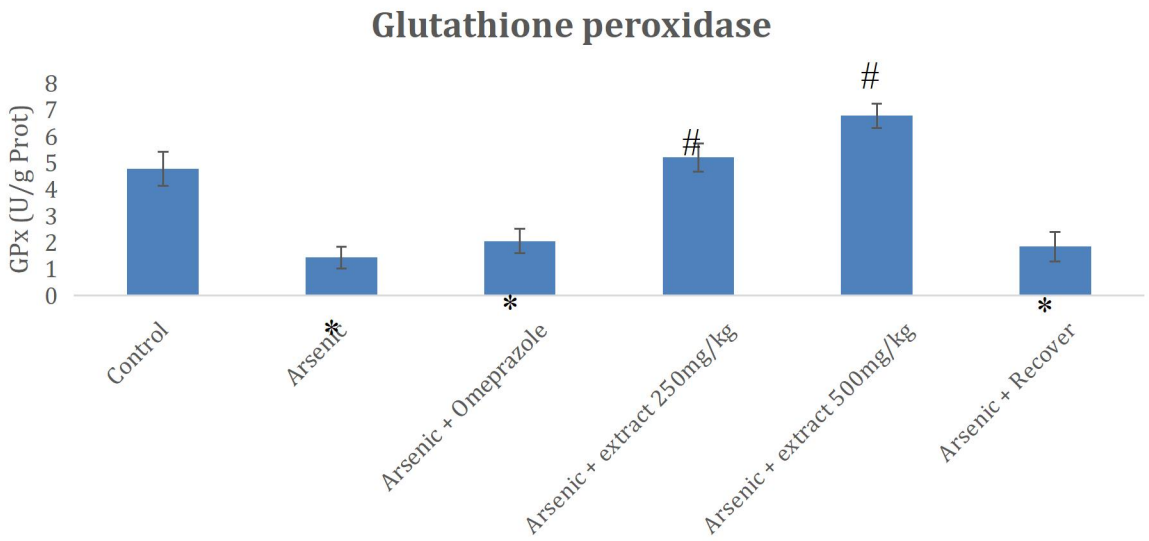


Figure 4.6: Chart showing Glutathione peroxidase activity across the experimental groups. Values are given as Mean ±SEM. * p <0.05 compared with control group; # p<0.05 compared with Arsenic only group.

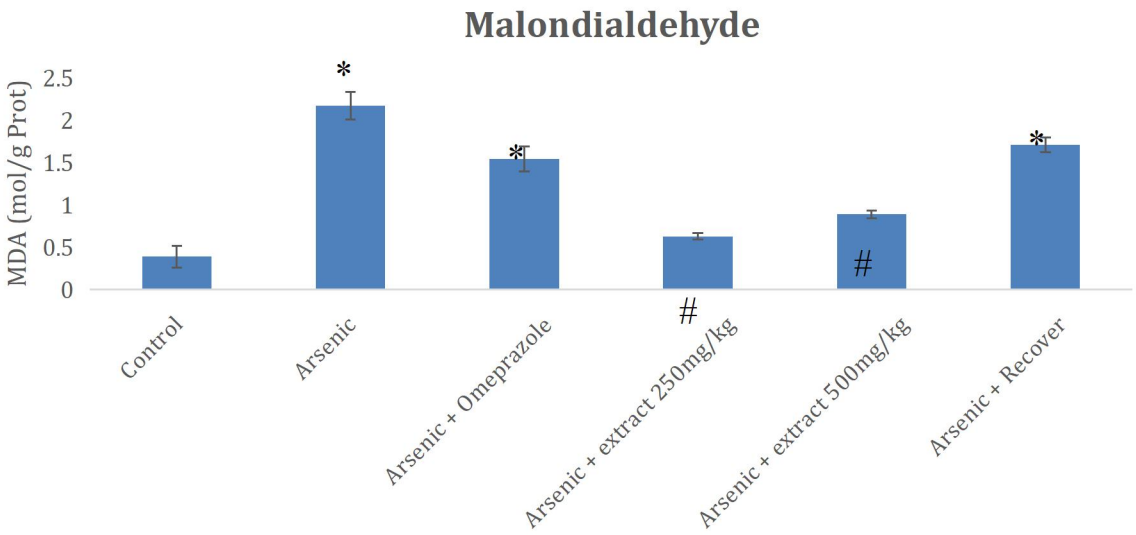


Figure 4.7: Chart showing Malondialdehyde concentration across the experimental groups. Values are given as Mean ±SEM. * p <0.05 compared with control group; # p<0.05 compared with Arsenic only group.

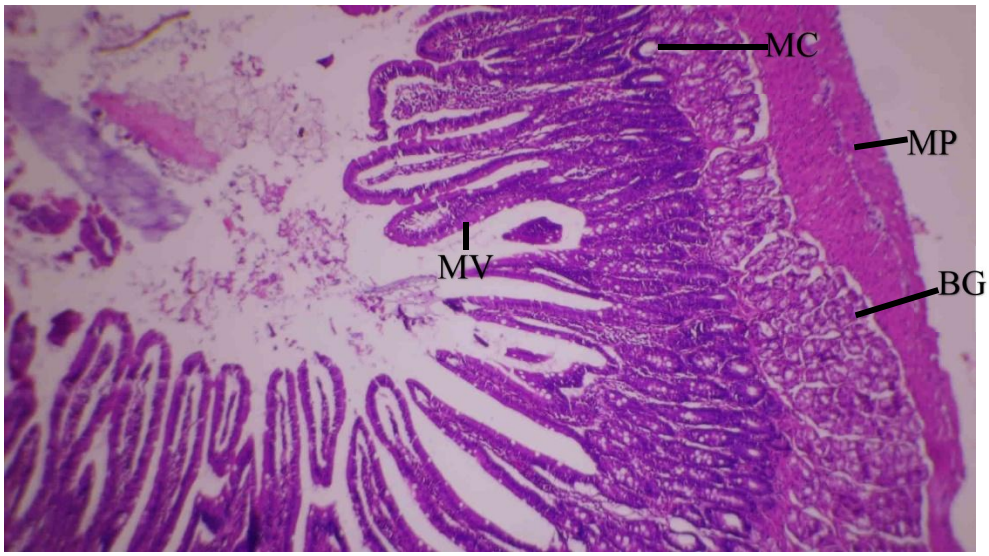


Plate 1. Rat duodenum, control, composed of: mucosal villi (MV), crypts (MC), muscularis propria (MP) and Brunner's glands (BG): H&E 40 X

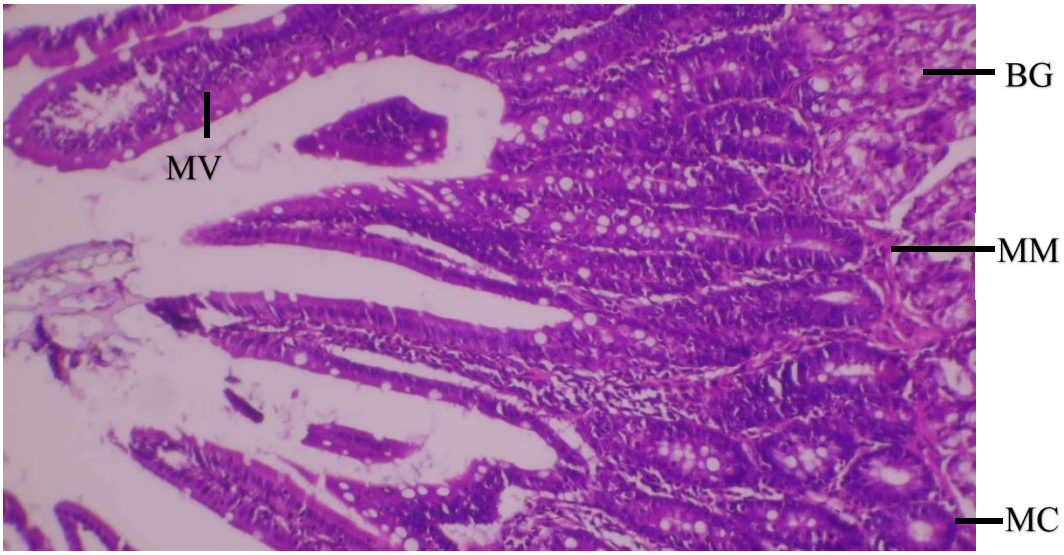


Plate 2. Rat duodenum, control, composed of: mucosal villi (MV), crypts (MC), muscularis mucosa (MM) and Brunner's glands (BG): H&E 100 X

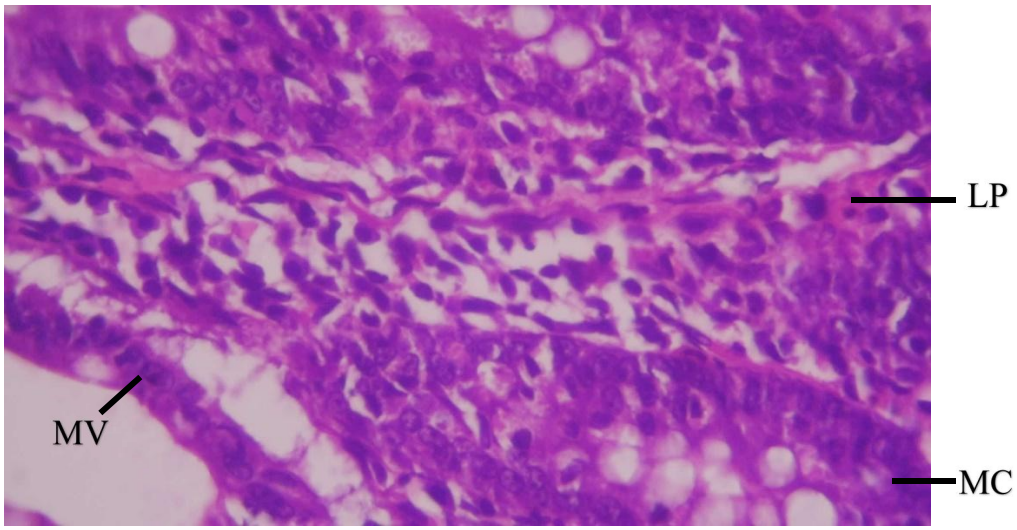


Plate 3. Rat duodenum, control, composed of: mucosal villi (MV), crypts (MC) and lamina propria (LP): H&E 400 X

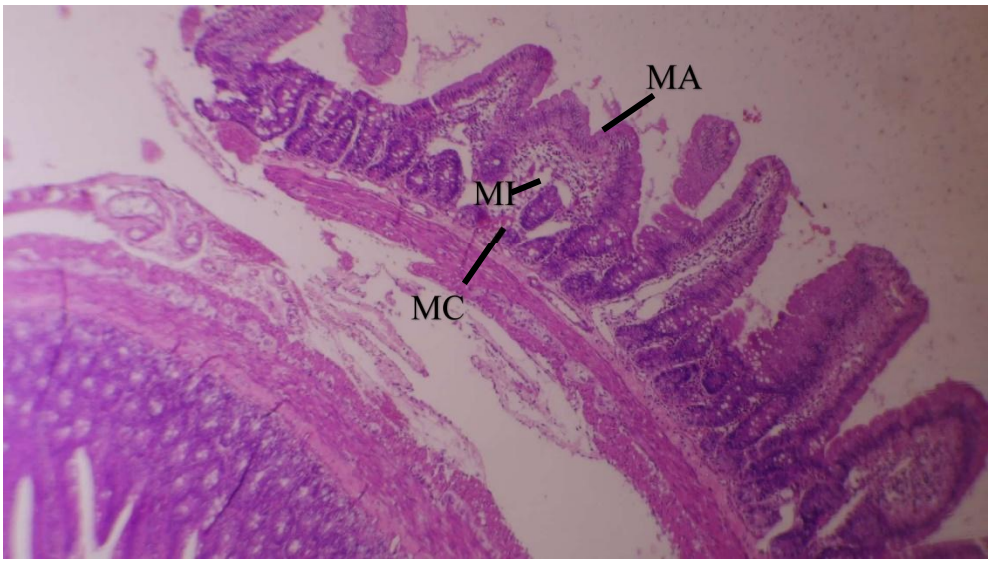


Plate 4. Rat duodenum given Arsenic only: show severe mucosal atro -phy (MA), mucosal infiltrates of inflammatory cells (MI) and mucos al congestion (MC):H&E 40 X

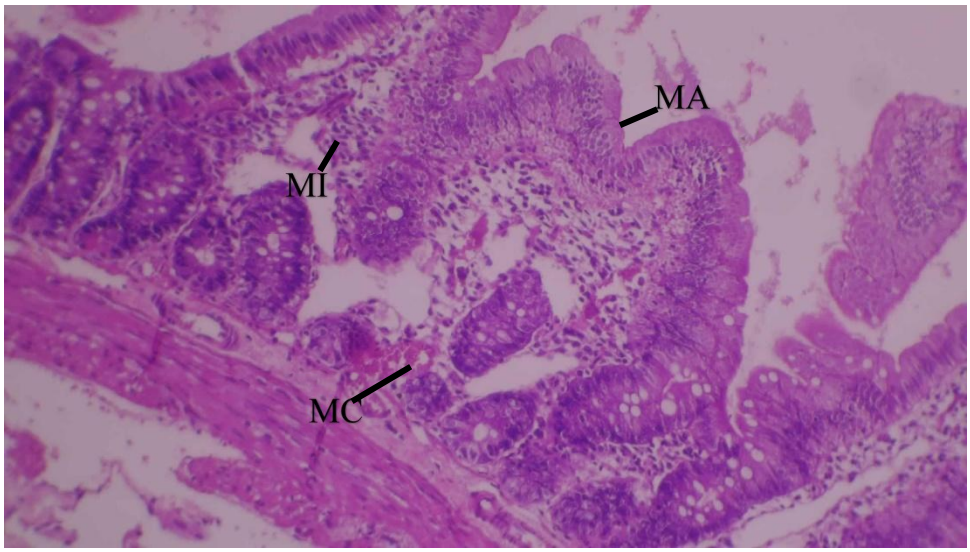


Plate 5. Rat duodenum given Arsenic only: show severe mucosal atro -phy (MA), mucosal infiltrates of inflammatory cells (MI) and mucos al congestion (MC)

: H&E 100 X

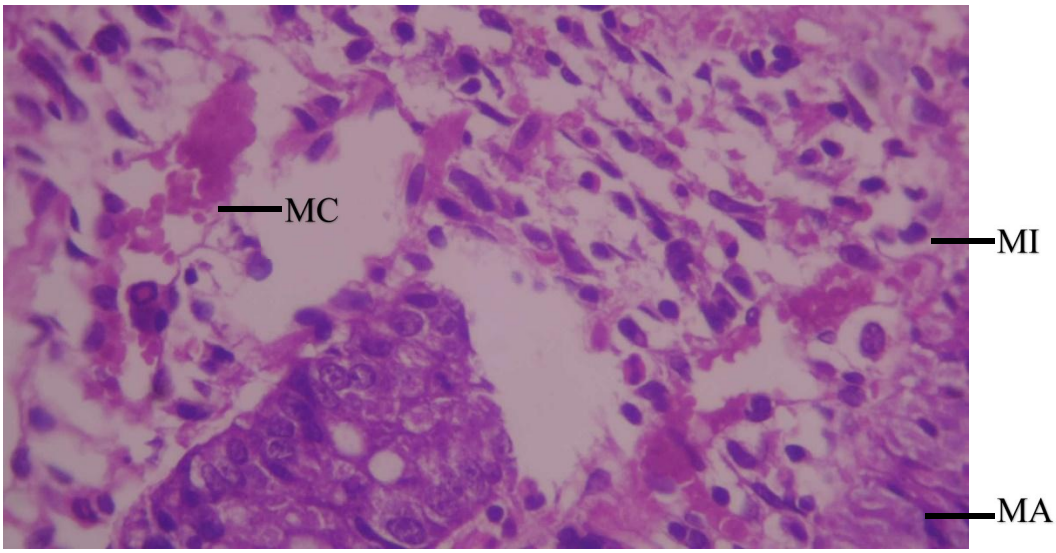


Plate 6. Rat duodenum given Arsenic only: show severe mucosal atro -phy (MA), mucosal infiltrates of inflammatory cells (MI) and mucos al congestion (MC): H&E 400 X

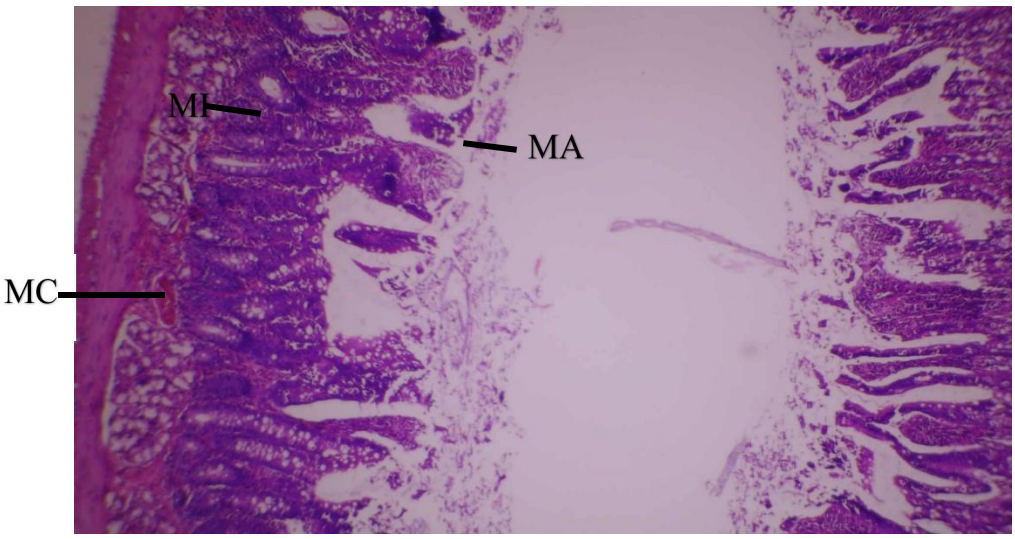


Plate 7. Rat duodenum given Arsenic + Omeprazole: mild mucosal atrophy (MA), mucosal infiltrates of inflammatory cells (MI) andmucosal congestion (MC): H&E 40 X

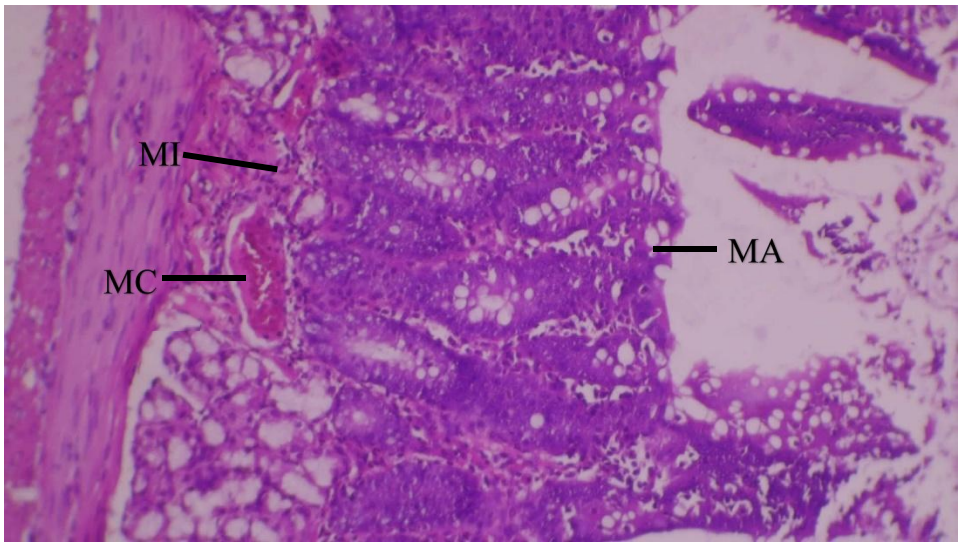


Plate 8. Rat duodenum given Arsenic + Omeprazole: mild mucosal atrophy (MA), mural infiltrates of inflammatory cells (MI) and mural congestion (MC): H&E 100 X

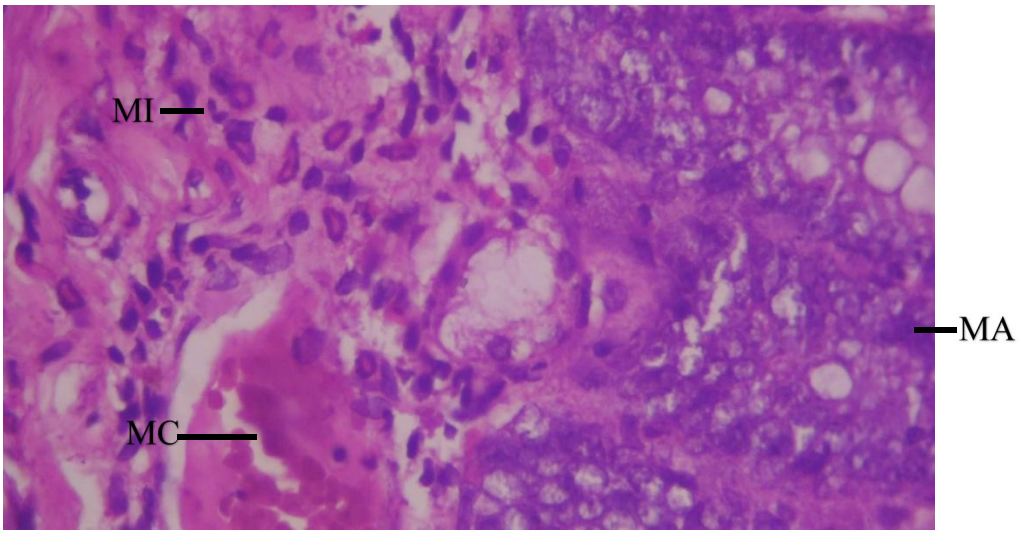


Plate 9. Rat duodenum given Arsenic + Omeprazole: mild mucosal atrophy (MA), mural infiltrates of inflammatory cells (MI) and mural congestion (MC): H&E 400 X

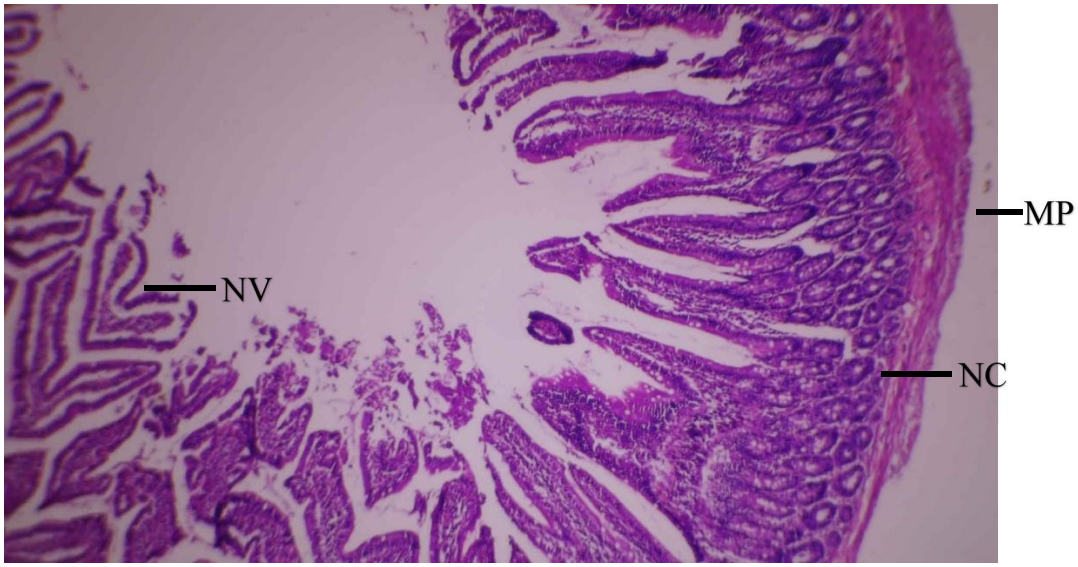


Plate 10. Rat duodenum given Arsenic + low dose extract show: normal mucosal villi (NV), mucosal crypts (NC) and muscularis propria (MP): H&E 40 X

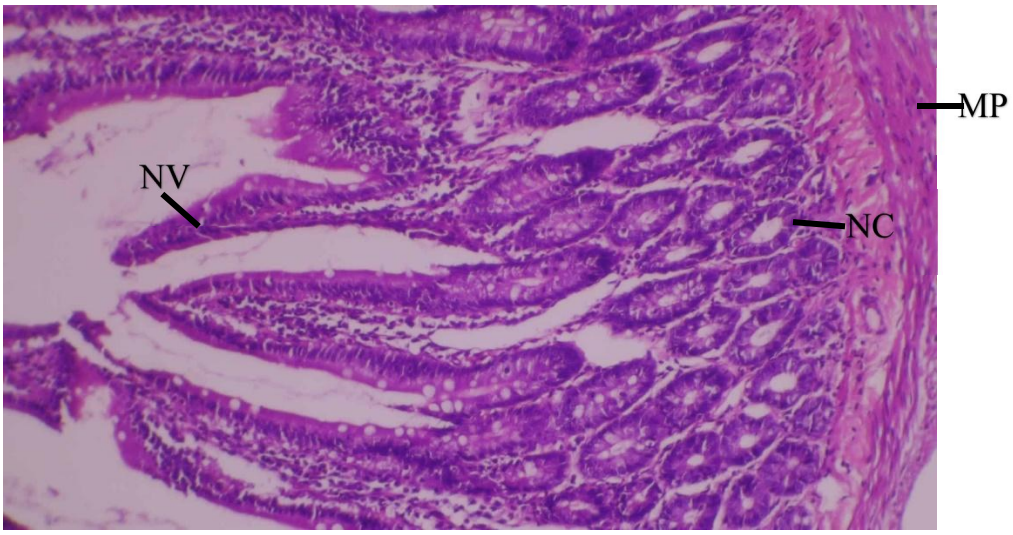


Plate 11. Rat duodenum given Arsenic + low dose extract show: normal mucosal villi (NV), mucosal crypts (NC) and muscularis propria (MP) : H&E 100 X

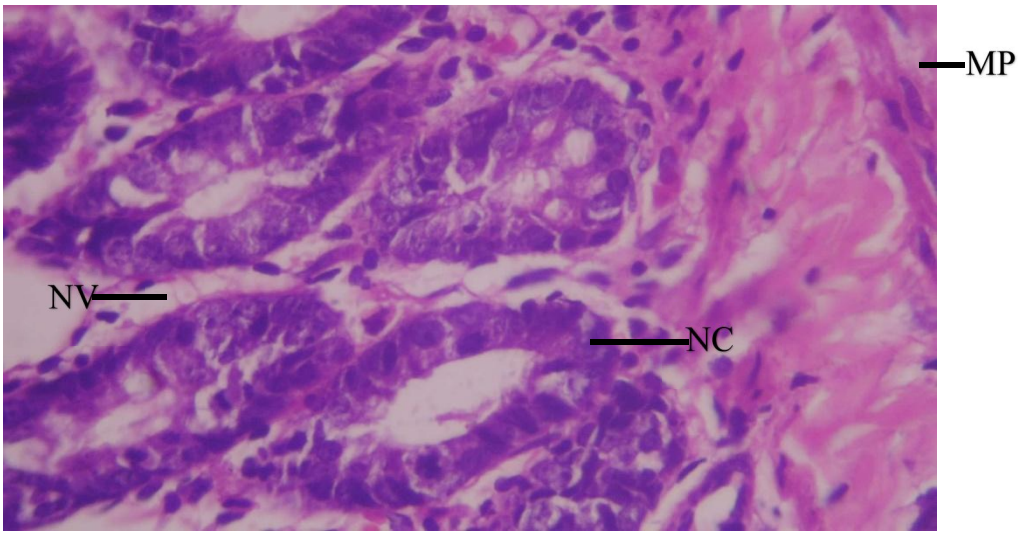


Plate 12. Rat duodenum given Arsenic + low dose extract show: normal mucosal villi (NV), mucosal crypts (NC) and muscularis propria (MP): H&E 400 X

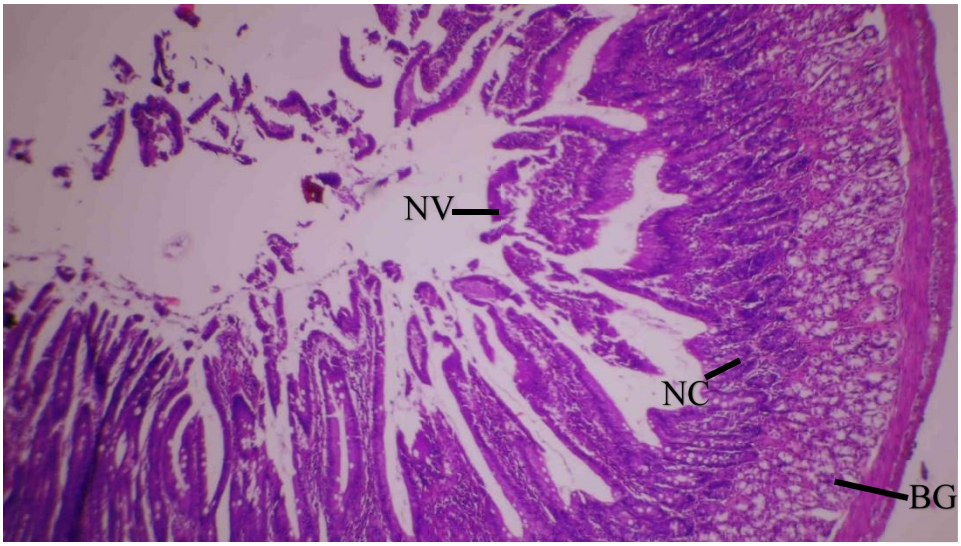


Plate 13. Rat duodenum given Arsenic + high dose extract show: normal mucosal villi (NV), mucosal crypts (NC) and Brunner's glands (BG): H&E 40 X

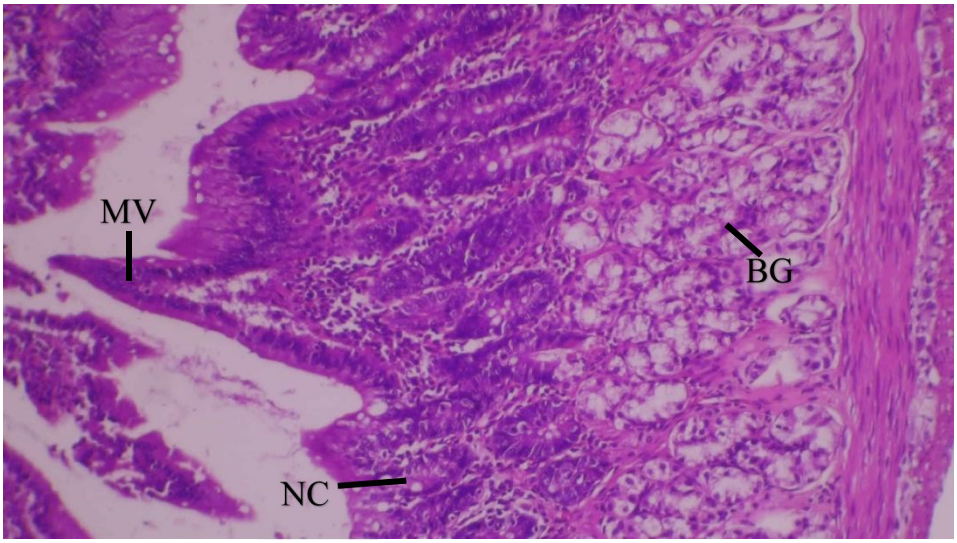


Plate 14. Rat duodenum given Arsenic + high dose extract show: normal mucosal villi (NV), mucosal crypts (NC) and Brunner's glands (BG): H&E 100 X

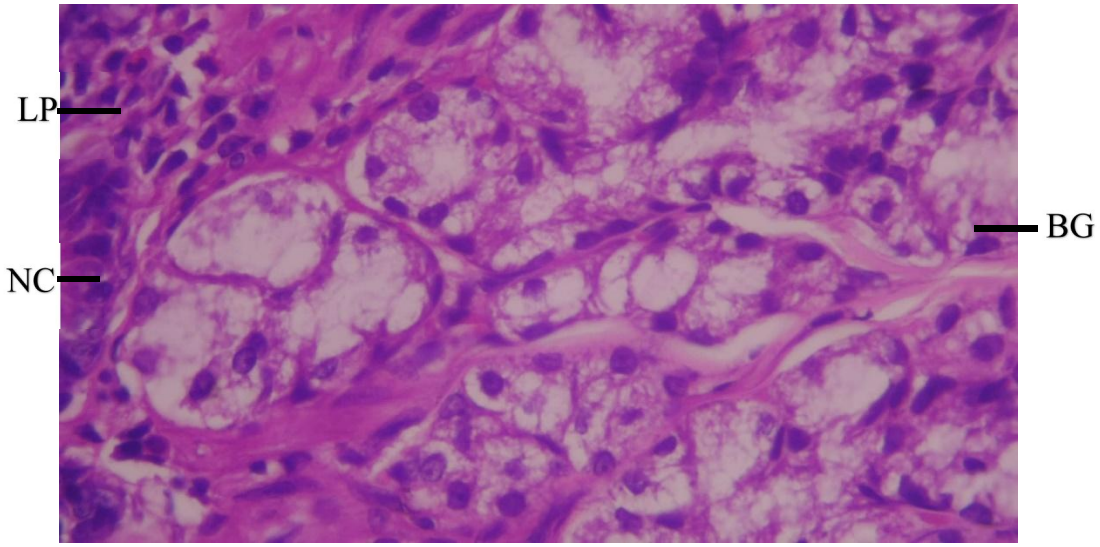


Plate 15. Rat duodenum given Arsenic + high dose extract show: normal Lamina propria (LP), mucosal crypts (NC) and Brunner's glands (MP): H&E 400 X

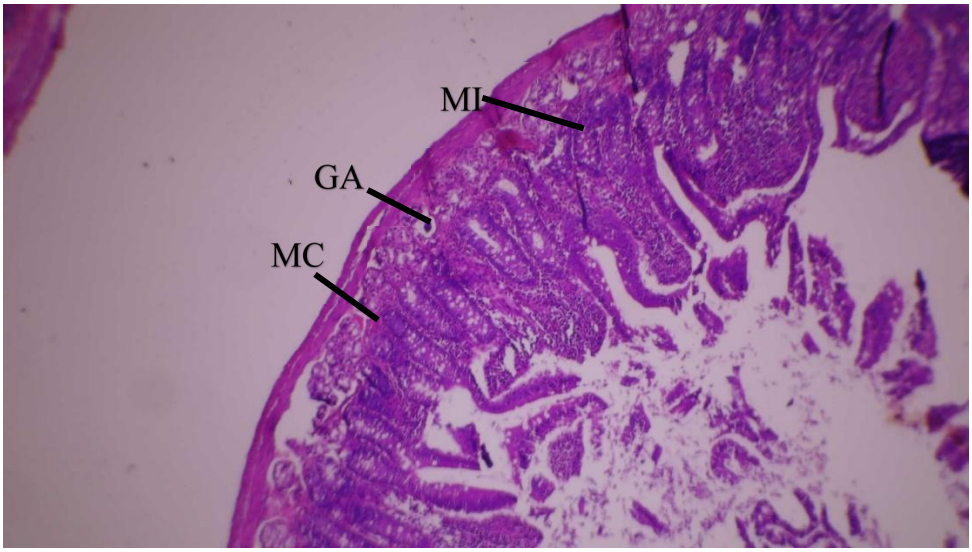


Plate 16. Rat duodenum given Arsenic only (Reversal) show: heavy mucosal infiltrates of inflammatory cells (MI), crypt atrophy (CA) and mucosal congestion (MC): H&E 40 X

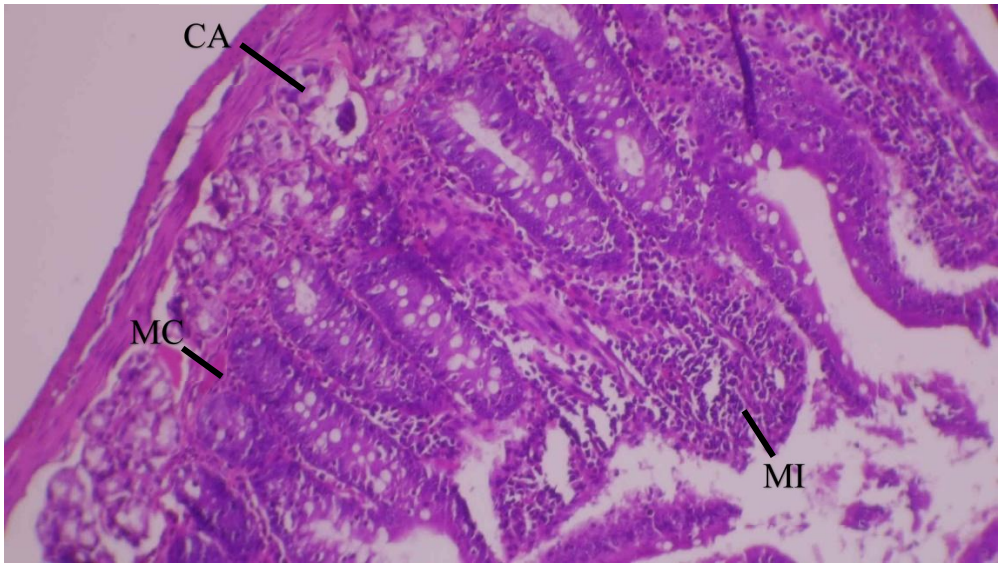


Plate 17. Rat duodenum given Arsenic only (Reversal) show: heavy mucosal infiltrates of inflammatory cells (MI), crypt atrophy (CA) and mucosal congestion (MC): H&E 100 X

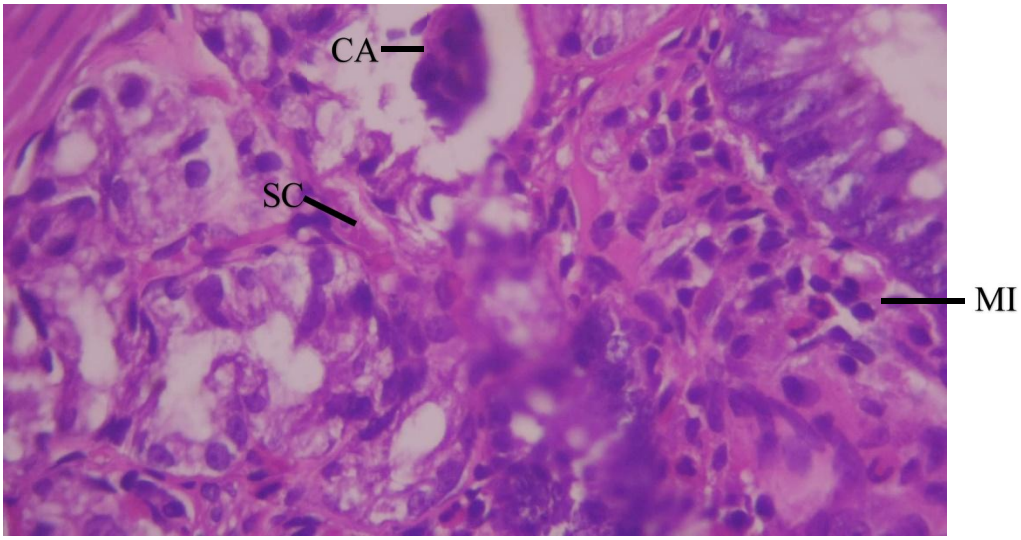


Plate 18. Rat duodenum given Arsenic only (Reversal) show: heavy mucosal infiltrates of inflammatory cells (MI), crypt atrophy (CA) and submucosal congestion (MC): H&E 400 X

CHAPTER FIVE

5.1. DISCUSSION OF FINDINGS

The result of this study showed that there was a significant decrease in the body weight of rats in the group administered arsenic trioxide only compared to the control group, which corresponds to previous research showing that arsenic trioxide exposure can suppress weight gain in rats(Liu et al.2021).In group D, the rats administered 250mg/kg aqueous leaf extract of *camellia sinensis* showed a significant increase in the body weight compared to groups administered arsenic trioxide only(group B).This suggests a protective or restorative role of green tea against arsenic trioxide-induced. According to Hijazi et al. (2015) reported that administration of green tea extract alone to adult Wistar rats did not cause any significant change in body weight, which showed that green tea does not promote weight gain, but help promote the arsenic-induced weight loss.

Result obtained from the group which was administered arsenic trioxide only showed a decrease in the level of SOD, CAT, GPX, compared to the control group. This corresponds with previous research showing that arsenic trioxide can impair antioxidant enzyme systems, leading to oxidative stress (Varghese et al. 2013). Result obtained from group C showed a decrease in the level of SOD, CAT, GPX, this could mean that omeprazole was not strong enough to the repair the antioxidant damage or the dose of omeprazole used (500mg/kg) was too high or not suitable to restore SOD level.

Result obtained showed that there was a significant increase in the level of SOD, CAT, GPX in group D and group compared to the arsenic trioxide group. This finding demonstrates that *camellia sinensis* can protect intestinal tissues from arsenic-induced oxidative damage. This result is in line Acharyya et al. (2015) which reported that green tea extract significantly restored the antioxidant enzymes, highlighting its protective antioxidant

properties. The result obtained showed there was a decrease in the level of SOD, CAT and GPX in group D which could be that the duodenal tissue may have accumulated arsenic trioxide or sustained damage.

Results obtained showed that there was a significant increase in the level of MDA in group B compared to the control indicating increased lipid peroxidation and oxidative damage. This finding corresponds with Acharyya et al. (2015), who reported that arsenic exposure significantly decreased intestinal SOD and catalase activity while at the same time increasing MDA in small intestinal tissues. Result obtained showed that there was an increase in the level of MDA in group C compared to the control. This could be that the antioxidant defense system was not fully restored. Result obtained showed that there was a decreased in the level of MDA in group D and E, compared to the arsenic group. This result is in line to previous findings, which reported that green tea can reduce MDA level and lower lipid peroxidation in rats, showing its protective effect against oxidative damage (Azeez et al. 2021). The result obtained showed that there was a significant increase in the level of MDA in group F. This could be as a result decreased antioxidant enzymes and higher level of lipid peroxidation.

Sections of the duodenum taken from rats given baseline feed and water freely show normal tissue architecture with well-defined mucosal villi, crypts, lamina propria, muscularis mucosa and submucosa. Similar findings were observed in sections taken from rats treated with Arsenic trioxide followed with graded doses of the extract.

Sections taken from rats treated with Arsenic trioxide only show severe mucosal atrophy, heavy mural infiltrates of inflammatory cells and mural congestion. These changes are lesions found in inflammation of the duodenum also called duodenitis (enteritis), similar findings were observed in sections taken from rats treated with Arsenic followed with

omeprazole (milder degree) and sections taken from rats from the reversal group to a comparable degree.

Thus Arsenic trioxide induced chronic duodenitis in adult Wistar rats, which was resolved by treatment with aqueous leaf extract of *Camellia sinensis*. However, omeprazole failed to resolve the lesions induced by arsenic in the duodenum, while the group treated with Arsenic trioxide and allowed to recover on their own failed to resolve.

5.2. CONCLUSION

This study investigated the effects of aqueous leaf extract of *Camellia sinensis* (green tea) on arsenic-induced duodenal damage in adult Wistar rats. The findings of this research demonstrate that arsenic trioxide administration can disrupt intestinal epithelial integrity, reduces villus height, induce crypt hyperplasia, oxidative stress and causes duodenal mucosal. But treatment with aqueous green tea leaf extract was found to greatly ameliorate these effects, improving the epithelial integrity, increasing villus height, and decreasing oxidative stress in the duodenal lining. The most important findings of this study show that arsenic trioxide exposure resulted in severe damage to the duodenal lining, as evidenced by inflammation and oxidative stress. Treatment with aqueous leaf extract of green tea decreased inflammation, oxidative stress, improved the epithelial integrity in the duodenal lining significantly. The anti-inflammatory and antioxidant activity of green tea leaf extract would probably account for the protective effect of green tea extract on arsenic trioxide-induced duodenal injury. The result of this research is applicable to the prevention and therapy of arsenic trioxide-induced duodenal damage. The result indicates that aqueous leaf extract of green tea could be a useful treatment in preventing arsenic trioxide exposure-caused adverse effects on the duodenum.

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