EFFECT OF AQUEOUS EXTRACT OF *Cyperus esculentus* ON ARSENIC TRIOXIDE-INDUCED INTESTINAL DAMAGE IN ADULT WISTAR RATS

BY

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TO

DEPARTMENT OF ANATOMY

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

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CERTIFICATION

This is to certify that this work was carried out under my supervision by PAUL OJOHRUMI FAVOUR (Matriculation Number: BMS2209309) of the Department of Anatomy, School of Basic Medical Sciences. University of Benin, Benin City, in partial fulfilment of the award of Bachelor of Science Degree (B.Sc.)

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EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this work to God Almighty for his mercy, grace, favour, provision and strength throughout my undergraduate programme.

ACKNOWLEDGMENT

First and foremost, I acknowledge Almighty God for seeing me through the time it took to complete this work, without whom it wouldn't have been possible.

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Lastly, to my wonderful colleagues, love you all and I wish you all the best in life.

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ABSTRACT

Arsenic trioxide (As_2O_3) is an environmental toxicant and a chemotherapeutic agent, whose efficacy is shadowed by its significant adverse effects on the gastrointestinal system. The large intestine, or colon, is a primary target for its toxicity, experiencing a cascade of damage from the cellular to the organ level. The aim of this study was to determine the effect of aqueous extract of *Cyperus esculentus* against arsenic trioxide induced damage on the large intestine across group of adult Wistar rats. 20 adult wistar rats were divided into 4 groups, each group containing 5 rats. Group A served as control while B, C, and D served as treatment groups. The wistar rats were fed with grower mash feeds and the rats had free access to water throughout the entire study period. Group A was the control and was administered 1ml of distilled water. Group B was administered Arsenic trioxide only (10mg). Group C was administered Arsenic trioxide (10mg) and a low dose of *Cyperus esculentus (200mg).* Group D also received arsenic trioxide (10mg) and a high dose of *Cyperus esculentus* (400mg). At the end of the administration period, the rats were anesthetized using chloroform. The large intestine was harvested and weighed and immediately fixed in formal-saline to avoid autolysis and taken through tissue processing. The result shows that, the control group (group A) showed normal large intestinal architecture. The Group B was given Arsenic trioxide only produced extensive mucosal necrosis and inflammatory bodies. Group B was observed extensive mucosal necrosis and heavy mucosal infiltrates of inflammatory bodies without much difference from Group C. Group C was better than Group B with some observable difference. Group D gave the best observable difference and results of all having ameliorated the harmful effects of Arsenic trioxide. There was a significant decrease of final weight in Group B. There was a significant increase in the gastrointestinal weight of Group D. Across the group there was an increase in the final weight of experimental animal when compared to their initial weights except for Group B. Therefore, *Cyperus esculentus* appears to effectively mitigate the harmful effects of arsenic trioxide exposure, offering a promising therapeutic approach for reducing mucosal damage and inflammation.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

The tiger nut, also known as the “underground walnut”, grows all over the world because of its high yield and broad prospects for comprehensive utilization. The tiger nut is the tiny tuber of *Cyperus esculentus*, which can be roasted and used to be sweetmeat in Egypt (Rubert, J. *et al.,* 2011). Moreover, it is an important representative crop of the Spanish Mediterranean region, with an annual production of 9000 metric tons (Sánchez-Zapata, E. *et al.,* 2012). Later, it was made into a refreshing drink called “horchata de chufa” with the appearance of dairy look in the Mediterranean area, which is usually consumed in summer (Rubert, J. *et al.,* 2011). At present, the popularity of “horchata” has been extended from Spain to the United States, France, the United Kingdom, Portugal, China and other countries (Sánchez-Zapata, E. *et al.,* 2012).

Although tiger nuts are widely cultivated around the world, the research on them is insufficient, which greatly limits their application (Adejuyitan, J.A. 2011). It has many nutrients that can be deeply explored and contains 22.14–44.92% lipids, 3.28–8.45% proteins, 23.21–48.12% starch, 8.26–15.47% fibers and 1.60–2.60% ashes (Adel, A.A.M. *et al.,* 2015). In addition, it contains bioactive substances such as organic acids, alkaloids and phenols (Nina, G.C. *et al.,* 2019). The tiger nut is a good source of edible oils that contain a lot of monounsaturated fatty acids. The nutritional value of tiger nut oil is similar to olive oil (Roselló-Soto, E. *et al.,* 2018). It also contains a lot of starch a renewable and low-cost food ingredient (dos Santos Silveira Junior, J.F. 2020). The content of protein is relatively small, but it is found to be suitable for diabetic patients or those with digestive dysfunctions and may prevent heart disease after

consumption (Ogunlade, I. et al., 2015). The dietary fiber in this tuber is effective in the prevention of colon cancer, obesity and gastrointestinal disorders (Viuda-Martos, M. *et al.,* 2010). Due to the presence of flavonoids, the tiger nut has good antioxidant properties and can be used as a source of natural antioxidants (Jing, S. et al., 2015).

Arsenic trioxide (As_2O_3) is an environmental toxicant and a chemotherapeutic agent, whose efficacy is shadowed by its significant adverse effects on the gastrointestinal system. The large intestine, or colon, is a primary target for its toxicity, experiencing a cascade of damage from the cellular to the organ level. This oxidative stress leads to inflammation, cellular apoptosis, and mucosal degeneration in the intestinal lining. As a result, individuals exposed to arsenic trioxide may experience a range of gastrointestinal symptoms, including diarrhea, abdominal pain, and impaired nutrient absorption (Shakoori et al., 2022). These effects are exacerbated by the generation of ROS, which further damages intestinal cells, impairing their ability to maintain the integrity of the mucosal barrier (Shakoori et al., 2022).

1.2 AIM OF STUDY

The aim of this study was to determine the effect of aqueous extract of *Cyperus esculentus* against arsenic trioxide induced damage on the large intestine across group of adult Wistar rats.

1.3 SPECIFIC OBJECTIVES OF THE STUDY

- **To evaluate the histopathological changes**
- **To determine the levels of oxidative stress markers**

- **To assess the inflammatory response**
- **To compare the intestinal morphology and biochemical parameters**
- **To determine the dose-dependent effects**

1.4 JUSTIFICATION OF STUDY

The proposed research topic is highly justified due to a critical intersection of pressing global and local public health concerns, significant gaps in existing scientific knowledge, and the promising yet underexplored therapeutic potential of a readily available indigenous food crop. The gastrointestinal tract is a primary route of arsenic exposure and a major site of its initial metabolism and absorption.

CHAPTER TWO

LITERATURE REVIEW

2.1 PLANT OF STUDY

Tiger nut (*Cyperus esculentus*) is a creeping perennial plant which belongs to the sedge family (Cyperaceae). It is commonly found populating wet marshes as well as stream and pond edges, where it grows in rough tufts (Arafat *et al*., 2009; Coskuner *et al*., 2002). It is an ancient plant commonly propagated in West Africa and South Europe. It produces sweet tubers that are consumed freshly or roasted. It can also be processed to obtain juice for beverage production and as animal feed (Udeozor, 2012). The tasty tubers from the plant has a length of 1-2 cm, with asymmetrical shape when dry and egg-like or round shape upon getting soaked in water (Coskuner et al., 2002). This plant is naturalized and cultivated in many parts of the world including Spain, Turkey, Thailand, India, Sierra Leone, Ghana, Togo, Mali, Senegal, Niger and Nigeria (Oyedepo and Odoje, 2014; Asante *et al*., 2014). It is known by many other names including chufa, zulu nut, edible galingale, yellow nutgrass, yellow nutsedge, earth chestnut, rush nut, edible rush as well as ground almond. Tiger nut is known by several names in Nigeria based on the various ethnic groups such as ‘aya’ (Hausa), ‘aki-Hausa’ (Igbo) and ‘ofio’ (Yoruba). (Bamishaiye *et al*., 2010; Adejuyitan, 2011; Ekeanyanwu and Ononogbu, 2010). Three varieties of the plant grow in Nigeria - the brown, black and yellow varieties. The yellow variety is larger in size, more attractive in colour and fleshy (Chukwuma *et al*., 2010). Tiger nut consumption is regarded in some countries as treatment for dysentery, indigestion, flatulence and mouth ulcer.

Additionally, there are claims that regular consumers of tiger nut might not suffer health complications such as diabetes, cardiovascular diseases, hyperlipidemia, prostate cancer, colon cancer, hernia, fibrosis, and abnormal menstruation. It also improves fertility in both male and female (Government of Ghana official portal). This review attempts to integrate available information on the chemical composition and pharmacological potential of tiger nut. Its application in the food, pharmaceutical and agricultural industries is also espoused.

Figure 1: Representation of Tiger nut

Source: specialtyproduce.com/produce/Tiger_Nuts_12905.php

2.1.1 Geographical Location

Tiger nuts (*Cyperus esculentus* L.) are little sweet tubers produced in the roots of an edible perennial grass-like plant, which is widely found around the Mediterranean countries (Coşkuner et al., 2002). These tubers are rich in energy (~400–450 kcal/100 g), due to their content in starch and fat (26–30% and 21–25%, respectively). Major fatty acids of tiger nut oil are monounsaturated (>60%), which is why its profile is comparable to olive or hazelnuts oils (Dubois et al., 2007). Tiger nuts also present a good percentage in protein (3–8%) and fiber (8–10%), as well as in vitamins (E and C) and minerals (phosphorous and potassium) (Adejuyitan, 2011, Arafat *et al*., 2009, Bosch *et al*., 2005, Chinma *et al*., 2009, Chukwuma *et al*., 2010, Tigernuts Traders, 2014). Owing to their composition, different studies have pointed the suitability of these tubers for diabetics and people with digestive disorders, and also in the prevention of heart diseases (Borges *et al*., 2008, Chukwuma *et al*., 2010, Tigernuts Traders, 2014). For this reason, these tubers are attracting industry interest, and the development of new tiger nut derived products has already been reinforced. These tubers are sold as snack food, and their flour has also been successfully used in bakery, in by-meat products and as a flavoring agent in ice cream production (Sánchez-Zapata *et al*., 2012). However, tiger nuts are mainly used in the production of tiger nut drinks, non-alcoholic beverages that are traditionally made in Spain (*horchata de chufa*) and Africa (*kunnun* aya). Current studies have evidenced the importance of tiger nut characteristics in the production of these quality derived products in terms of their physicochemical characteristics and nutritional profile, but despite most of these tubers being characterized and submitted to the sanitary official controls, the quality characteristics of some

batches are still in doubt (Addy and Teshola, 1984, Bosch *et al*., 2005, Ejoh and Ndjouenkeu, 2007, Oladele and Aina, 2007, Sánchez-Zapata *et al*., 2010).

Owing to all of this, the objective of the present work was to compare the quality characteristics of tiger nuts from different geographical origin which are commercialized in the European market for the production of derived products, in order to generate more information about the effect of site cultivation on their physico-chemical characteristics. Furthermore, another goal was to obtain tiger nut protein fractions and determine partially their chemical characteristics, data about their chemical characterization that remains scarcely explored.

2.1.2 Host/Plant Relationship of Tigernut

The tiger nut is not a nut but a small tuber produced on the rhizomes of *Cyperus esculentus L.*, a perennial sedge plant belonging to the Cyperaceae family **(Pascual *et al.*, 2000)**. The plant's relationship with its environment and cultivation system is complex, defined by its robust growth habits and specific soil requirements.

The plant is characterized by a basal rosette of long, grass-like leaves and triangular stems (culms) that can grow up to 60-90 cm in height. The tubers, the economically valuable part, form underground on slender rhizomes that radiate from the base of the plant. A single plant can produce hundreds of tubers, which serve as organs for vegetative propagation and nutrient storage **(De Castro *et al*., 2015)**. This prolific underground network is a key characteristic of its survival strategy.

Cyperus esculentus thrives in loose, well-drained, sandy loam soils, which allow for easy tuber formation and expansion. Heavy clay soils can hinder rhizome growth and lead to

malformed tubers **(Adejuyitan, 2011)**. The plant requires a warm climate with a frost-free growing season of about 4 to 5 months and prefers full sun exposure for optimal photosynthesis and yield **(Sánchez-Zapata *et al*., 2012)**.

A critical aspect of the tiger nut's "relationship" is its dual identity. While it is intentionally cultivated in regions like Spain (Valencia) and West Africa for its tubers, it is also classified as one of the world's most invasive and persistent weeds in other agricultural systems, such as corn and soybean fields **(Ghersa *et al*., 2000)**. Its success as a weed is directly linked to its extensive rhizome system and the resilience of its tubers, which can remain dormant in the soil seed bank for multiple years before sprouting, making it extremely difficult to eradicate **(Tumbleson & Kommedahl, 1961)**.

The plant is moderately nutrient-demanding. Adequate phosphorus and potassium are crucial for robust tuber development **(Okafor *et al*., 2003).** Furthermore, as part of its relationship with the soil ecosystem, the cultivation of tiger nut has been reported to positively impact soil structure. The dense root and rhizome system can help bind soil particles, reducing erosion, while the decomposition of its organic matter can contribute to soil fertility **(Chukwuma *et al*., 2010).**

2.1.3 CONSTITUENTS OF TIGER NUT (CYPERUS ESCULENTUS) EXTRACTS

The outcome of the proximate composition of 100 g of tigernuts showed the various components in a decreasing order of moisture, carbohydrate, crude fat, crude fiber, crude protein, and ash contents. This implies that tigernuts are high in moisture, carbohydrate, fat and fiber content. The mineral content of tigernuts in a decreasing order comprised of potassium (k), phosphorus (P), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), zinc (Zn), and copper (Cu) respectively. It was also shown to contain a high level of vitamin C and little

vitamin A content. They are fairly good sources of protein. Fresh tigernuts are high in moisture content. This indicates that it can perish easily due to microbial attack. Tigernuts could be eaten fresh as snacks by young and old (children, adolescents, and adults, pregnant and lactating mothers) for its high nutrients. These nutrients could significantly contribute to the body's metabolic processes, refreshing the body as well. Consequently, if 100 g of tigernuts is consumed, it could significantly contribute more than 40 % of carbohydrate to a child's (4-9 yrs) daily carbohydrate requirement and more than 32 % of carbohydrate to an adult's daily carbohydrate need (FAO/WHO/UNU, 2002). Tigernuts in comparison to other starchy roots and tubers such as sweet potatoes, yam, and cassava, have interestingly, significantly higher fat content and could contribute more than 73% of fat to a child's daily fat need and more than 49% of fat to an adult daily fat requirement (FAO/WHO/UNU, 2002). The fat content of tigernuts are relatively similar to that of nuts and seeds but are higher than that of cereals and compares well with that of soya beans (Sanchez-Zapata E *et al*., 2012). Tigernut fiber values from the findings are in line with the report of Umerie et al. (Umerie SC *et al*., 1997). In contrast, Temple et al., reported a lower fiber value (5.50%). With about 100 g of tigernuts if consumed, the fibre content could play an important role in the reduction of pressure and transit time of food through the body aiding in digestion (Temple VJ. *et al.*, 1998). Fibre aids in the alleviation of flatulence problem, thus, tigernut fibre could be explored in formulating diets for treating indigestion, constipation and non- communicable diseases such as colon cancer, diverticulosis, coronary heart disease and obesity (Wardlaw GM *et al*., 2002).

The protein content of tigernuts in this present study is in line with the range reported by Tiger nuts Traders et al. (Tigernuts traders, 2005). The Protein content of tigernuts is higher than that of most starchy roots and tuber crops such as cassava, sweet potatoes and yams (Sanchez-

Zapata E *et al.,* 2012). Tigernut's protein content compares well with that of cereals such as rice and sorghum (Ndubuise LC, 2009). Tigernuts can be a source of plant protein if it is bio-available. Tigernuts could contribute more than 17% of protein to adult's daily protein need and more than 26% to a child's daily protein requirement. About 100 g of tigernuts could provide about 15-22% of the energy required for children per day (4-9 yrs) and 8-16% of daily energy requirement for adolescents, adults and pregnant mothers (FAO/WHO/UNU, 2002). The ash content of 100 g of tigernut which was 1.18% fell within the range reported for other starchy roots and tubers such as yam, cassava, and potatoes. Tigernut's ash value was in line with the reports of Temple et al. (Temple VJ et al., 1998). However, values reported by Umerie et al., on tigernuts had significantly higher values (2.48% and 6.70% dry weight, respectively) (Umerie SC et al., 1997). Magnesium provides bone strength, aids enzyme, nerve and heart functions. Tigernuts could contribute adequate Mg and Zn (100%) to the daily need of children. Phosphorus enhances quick release of energy in the body and may combine with calcium for bone and teeth development. Tigernuts are relatively low in calcium and sodium. Recent studies on blood pressure showed that a diet rich in potassium and magnesium but low in sodium can lead to a decrease in blood pressure within days of beginning a specific diet (Wardlaw GM et al., 2002). Potassium aids nerve impulse transmission and it is a major cation of intracellular fluid. High potassium to low sodium ratio of tigernuts therefore, may be imperative in diet formulations for patients with high blood pressure and edema as well. Tigernuts contain protective nutrients because it could supply adequate zinc, copper, iron, vitamin C and E. Zinc is an integral part of hormones and more than nearly 100 different enzymes. Zinc is vital in several metabolic reactions and may play an important role in alcohol metabolism, immunity, sexual development, and reproduction. Copper aids in iron metabolism.

It works with many antioxidants, enzymes especially those involved in protein metabolism and hormone synthesis. The iron content of tigernuts could contribute to preventing anemia. Fe is the functional component of hemoglobin and other key compounds used in respiration, immune function, and cognitive development. The Fe content in tigernuts (100 g) could be enough to cover the daily minimum needs (providing about 67-68 %) for children. Tiger nuts could provide about 27-64% of adolescents or adults daily iron need and 18-49% of pregnant mother's daily iron needs.

If 100 g of tigernuts is eaten per day by children between 4-9 years old, the vitamin C content could be adequate, providing about 88-100% of their recommended dietary intake (FAO/WHO/UNU, 2002). Vitamin C is important in collagen, hormone and neurotransmitter synthesis. 100 g of tigernuts could also meet about 77% daily vitamin C needs of adolescents, 69% for adults and 52% for pregnant mothers. Besides, high vitamin C concentration in tigernuts may render soluble the iron content and make it more available (Gambo A *et al*., 2014). Vitamin C is antioxidants which are important in the prevention of coronary diseases and cancer (Wardlaw GM *et al.,* 2002).

2.1.4 TRADITIONAL/ETHNOBOTANICAL USES OF TIGERNUTS

In **West African** countries like Nigeria, tiger nuts have been a vital part of traditional diets for centuries. Known locally as **"aya"** or "aki hausa," they are consumed raw, roasted, or soaked. Tiger nuts are a popular street food, enjoyed for their satisfying crunch and natural sweetness. They are also used to make **kunu aya,** a creamy, spiced drink made by blending tiger nuts with dates, ginger, and spices. Beyond culinary use, tiger nuts have been employed in traditional medicine to treat digestive issues and boost fertility.

In the **Middle East**, tiger nuts have been valued for their energy-boosting properties. They were commonly consumed by travelers and laborers as a portable source of nourishment. Traditional Middle Eastern medicine recognized tiger nuts for their digestive and anti-inflammatory benefits. They were often used to soothe stomach ailments and support overall health. They have historically served as a vital source of energy and nutrition, especially during times of famine or long journeys, due to their durability and high energy content **(Bamishaiye *et al.*, 2011).**

2.1.5 TAXONOMY/CLASSIFICATION OF TIGERNUTS

Preferred scientific name: Cyperus esculentus

Preferred common name: Tigernut

Taxonomic Tree

Domain: **Eukaryota**

Kingdom: Plantae (plants)

Subkingdom: Viridiplantae

Phylum: Tracheophyta

Subphylum: Magnoliophytina

Class: Liliopsida

2.1.6 THE HEALTH BENEFITS OF TIGER NUTS

- **Promotes Digestive Health**

Tiger nuts are an excellent source of dietary fiber, with a content ranging from 8% to 10%. This high fiber content, particularly insoluble fiber, acts as a prebiotic, stimulating the growth and activity of beneficial gut bacteria such as *Lactobacillus* and *Bifidobacterium* **(Sánchez-Zapata et al., 2012)**. This promotes a healthy gut microbiome, which is crucial for efficient digestion, enhances bowel regularity, and may help alleviate issues like constipation and diarrhea **(Belewu & Belewu, 2007)**.

- **Supports Cardiovascular Health**

The lipid profile of tiger nut oil is highly beneficial for heart health. It is rich in monounsaturated fatty acids (MUFAs), primarily oleic acid, which constitutes over 70% of its total fat content. Diets high in MUFAs are well-established for their ability to help reduce levels of Low-Density Lipoprotein (LDL) or "bad" cholesterol while maintaining or increasing High-Density Lipoprotein (HDL) or "good" cholesterol, thereby improving the overall lipid profile and reducing the risk of atherosclerosis **(Dubois *et al*., 2007).** Furthermore, the presence of arginine, an amino acid that produces nitric oxide, helps in vasodilation, which can contribute to lower blood pressure **(Borges *et al*., 2008).**

- **Potent Antioxidant and Anti-inflammatory Effects**

Tiger nuts contain significant levels of antioxidant compounds, including vitamin E (tocopherols) and various phenolic compounds like p-coumaric acid and ferulic acid. These antioxidants help neutralize harmful free radicals in the body, reducing oxidative stress, which is a key contributor to chronic diseases, aging, and inflammation **(Codina-Torrella *et al*., 2015)**. The anti-inflammatory properties are further supported by the presence of oleic acid and phytosterols like β-sitosterol **(Eke *et al*., 2018).**

- **Beneficial for Blood Sugar Control**

The combination of high dietary fiber and a specific type of starch that is resistant to rapid digestion results in a low glycemic index for tiger nuts. The fiber slows down the absorption of sugars in the bloodstream, preventing sharp spikes in blood glucose and insulin levels after a meal. This makes tiger nuts a suitable dietary component for individuals with diabetes or those at risk of developing the condition **(Adejuyitan, 2011; Chinma *et al*., 2009)**.

- **Potential Aphrodisiac and Reproductive Health Properties**

One of the most renowned traditional uses of tiger nuts is as an aphrodisiac. Scientific studies have provided some support for this claim. Research on animal models has shown that tiger nut

extract can significantly increase sperm count, motility, and testosterone levels, suggesting a potential role in enhancing male fertility **(Okafor *et al*., 2014)**. This effect is often attributed to its rich content of arginine, zinc, and vitamin E, all of which are essential for reproductive health.

- **Boosts Immune Function and Overall Vitality**

The rich mineral content of tiger nuts, including high levels of phosphorus, potassium, magnesium, and zinc, contributes to various bodily functions, including nerve signaling, muscle contraction, and immune system support. Vitamins E and C further bolster the immune system's defenses. Traditionally, the tubers are consumed as a general tonic to combat fatigue and weakness, a use that is supported by their high energy and nutrient density **(Chukwuma *et al*., 2010)**.

2.1.7 POSSIBLE SIDE EFFECTS

Tiger nuts can be contaminated with toxic weeds, molds, and bacteria if not properly handled and stored. As tiger nuts are often grown in warm, humid climates, improper drying and storage conditions may lead to the growth of Aspergillus flavus, a toxic mold that produces aflatoxin. Aflatoxin is a carcinogenic compound that can cause liver damage and suppress the immune system. Other contaminants like Salmonella and E. coli have also been detected in tiger nuts. Tiger nuts can interfere with certain medications you may be taking. As with any food, check with your doctor or pharmacist to ensure tiger nuts do not interact negatively with any prescription or over-the-counter drugs you are on. Tiger nuts may cause allergic reactions in some people, especially those with nut allergies. This is because tiger nuts are tubers that are closely related to nuts despite being called “nuts.” The proteins found in tiger nuts can trigger a

reaction in people with nut allergies, ranging from minor irritation to a life-threatening anaphylactic shock. Be very cautious if you have any known nut allergies. Cases of **anaphylaxis** have been reported, suggesting that it is a potential, albeit uncommon, allergen. Individuals with known food allergies should exercise caution when trying tiger nuts for the first time **(Asero *et al*., 2009)**.

2.2 ARSENIC TRIOXIDE

Arsenic Trioxide is an inorganic compound with the formula (As_2O_3) in which the oxidation state is +3. It a white, crystalline solid or a fine white powder**. It is notoriously tasteless and odorless. It is also known as** white arsenic, Arsenous oxide, Arsenous anhydride and Diarsenic trioxide. It is sparingly soluble in water but dissolves readily in acids and alkalis. **Arsenic trioxide is profoundly toxic and dangerous. It has various application such as medical us, historical and industrial uses and is used as a reagent** in chemistry, particularly in analytical chemistry and organic synthesis. However, due to its extreme toxicity, its use is now specialized and handled with extreme caution, often replaced by safer alternatives when possible.

Figure 2: Arsenic trioxide

Source: https://www.indiamart.com/proddetail/arsenic-trioxide-powder-24793025491.html

Empirical formula As_2O_3

Molecular Formula As_2O_3

IUPAC name **Arsenic (III) oxide**

Other name includes white arsenic, Arsenous oxide, Arsenous anhydride and Diarsenic trioxide

2.2.1 STRUCTURE OF ARSENIC TRIOXIDE.

Arsenic trioxide is a chemical compound made of arsenic and oxygen, with the molecular and empirical formula As_2O_3. It is a white, toxic solid that exists in both crystalline and amorphous forms. In its crystalline form, each arsenic atom is bonded to three oxygen atoms in a pyramidal shape. These atoms are linked together by As–O–As bridges, forming a network-like structure.

Arsenic trioxide is primarily a covalent compound, and does not form ions in its solid state. However, when dissolved in water, it can produce Arsenite ions such as AsO_3^{3-}. This behavior shows that it can act as an acid in aqueous solutions. In arsenic trioxide, arsenic is in the +3 oxidation state, while oxygen is in the −2 oxidation state.

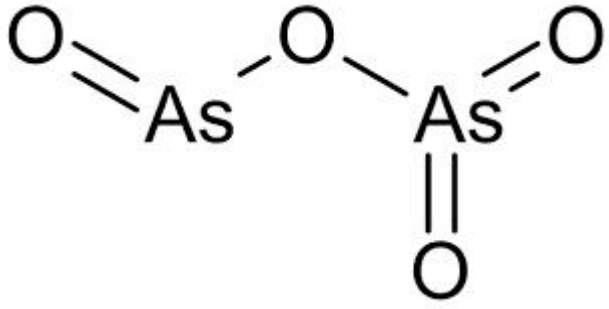

Figure 3: structure of Arsenic trioxide

2.2.2 PROPERTIES OF ARSENIC TRIOXIDE

- **Physical properties**

Arsenic trioxide (As_2O_3) is a white, odorless solid that can appear either as a fine powder or in crystalline form. At room temperature, it remains a stable solid with a monoclinic crystal structure. When heated, arsenic trioxide turns yellow but returns to white upon cooling a reversible color change that is one of its noticeable physical characteristics.

The compound has a molar mass of approximately 197.84 g/mol and a density of around 3.74 g/cm^3 in its crystalline state. It melts at about 312 °C and boils at roughly 465 °C. However, instead of boiling like many other compounds, arsenic trioxide tends to sublime, transitioning directly from solid to gas under certain conditions.

Arsenic trioxide is only slightly soluble in water, with a solubility of about 20 grams per liter at 25 °C. This limited solubility is enough to allow it to form weak acidic solutions when dissolved, such as arsenious acid (H_3AsO_3).

Despite its chemical usefulness in industries like glassmaking and electronics, arsenic trioxide is highly toxic and poisonous. Handling it requires strict safety measures due to its dangerous health effects, especially when inhaled or ingested.

- **Chemical Properties**

Arsenic trioxide (As_2O_3) is a chemically reactive compound, particularly known for its acidic and toxic nature. One of its key chemical properties is that it behaves as an acidic oxide. When dissolved in water, it forms arsenious acid (H_3AsO_3), a weak acid:

$$As_2 \quad O_3 \quad +3H_2 \quad O \rightarrow 2H_3 \quad AsO_3$$

It also reacts with strong **bases** like sodium hydroxide (NaOH) to form **arsenite salts**. For example:

$$As_2 \quad O_3 \quad +6NaOH \rightarrow 2Na_3 \quad AsO_3 \quad +3H_2 \quad O$$

In terms of oxidation, the arsenic in As_2O_3 is in the **+3 oxidation state**. This makes it a **reducing agent** in some chemical reactions. However, under oxidizing conditions, it can be converted to **arsenic pentoxide (As_2O_5),** where arsenic is in the **+5 state**.

$$2As_2 \quad O_3 \quad +O_2 \quad \rightarrow 2As_2 \quad O_5$$

Arsenic trioxide is also known for its ability to react with halogens, acids, and certain metals. It dissolves in **hydrochloric acid (HCl)** and forms chlorinated arsenic compounds, showing its versatility in chemical synthesis.

2.2.3 SYNTHESIS OF ARSENIC TRIOXIDE

1. From Arsenic Metal:

When **pure arsenic (As)** is heated in the presence of **oxygen (O_2)**, it burns and forms arsenic trioxide gas. This gas cools and becomes a white solid.

Reaction:

$$4As+3O_2 \rightarrow 2As_2 O_3$$

This is a simple reaction: arsenic combines with oxygen to make arsenic trioxide.

2. From Arsenic Ores (like Arsenopyrite):

Arsenopyrite (FeAsS) is a common ore containing arsenic. When it's **roasted (heated strongly with air)**, the arsenic and sulfur are oxidized, and arsenic trioxide is formed.

Reaction:

$$4FeAsS+11O_2 \rightarrow 2As_2 O_3 +2Fe_2 O_3 +4SO_2$$

- Arsenic (As) becomes **As_2O_3**
- Sulfur (S) becomes **SO_2**
- Iron (Fe) becomes **Fe_2O_3**

The arsenic trioxide comes out as a **gas** and is then **cooled and collected as a white solid**.

2.2.4 HISTORY OF ARSENIC TRIOXIDE

Arsenic itself was first identified by German alchemist Albertus Magnus in 1250, though its compounds were used and mined by ancient Chinese, Greek, and Egyptian civilizations much earlier. Arsenic, especially in the form of arsenic trioxide commonly called “white arsenic” has long held a dual reputation as both **poison** and **medicine**. Its story spans millennia, blending alchemy, medicinal breakthroughs, criminal cases, and modern cancer therapy. Evidence from **ancient Greece and Rome** shows arsenic compounds like orpiment (As_2S_3) and realgar (As_4S_4) were used medicinally for ulcers, plagues, and even as depilatories. For over **2,400 years**, arsenic was a staple of traditional remedies across civilizations, known for its potent effects.

2.2.5 USES OF ARSENIC TRIOXIDE

Arsenic trioxide (As_2O_3) is a highly toxic compound, but despite its poisonous nature, it has found a number of important uses in medicine, industry, and scientific research. Its applications have evolved over time, with many former uses now banned or restricted due to health and environmental concerns. However, its value in certain fields especially cancer treatment remains significant.

One of the most important modern uses of arsenic trioxide is in medicine, particularly in the treatment of acute promyelocytic leukemia (APL), a rare but serious type of blood cancer. Arsenic trioxide is the active ingredient in the drug Trisenox, which has been approved by the U.S. Food and Drug Administration (FDA). In cancer therapy, it works by inducing programmed cell death (apoptosis) in leukemia cells and helping to restore normal blood cell function. This has made it a life-saving treatment for many patients with APL.

Arsenic trioxide plays a role in various industrial applications. It is used in the glass and ceramics industry to remove bubbles and improve clarity in specialty glass products. It also serves as a refining agent in metallurgy, especially in the extraction and purification of precious metals such as gold and copper. Although once widely used in pigments and wood preservatives, these applications have largely been phased out due to the compound's toxicity.

In the field of scientific research and chemical manufacturing, arsenic trioxide is used as a starting material for synthesizing other arsenic compounds. It is also used in the production of certain semiconductors and optical materials.

2.2.6 MECHANISM OF ACTION OF ARSENIC TRIOXIDE

Arsenic trioxide causes morphological changes and DNA fragmentation characteristic of apoptosis in NB4 human promyelocytic leukemia cells *in vitro*. Arsenic trioxide also causes damage or degradation of the fusion protein PML/RAR-alpha. It is suspected that arsenic trioxide induces cancer cells to undergo apoptosis.

2.2.7 ADVERSE EFFECTS OF ARSENIC TRIOXIDE IN HUMNAN

Arsenic is a well know human toxicant and carcinogenic metalloid. Exposure to arsenic and its compounds can have adverse effects on human health (Tapio, S. *et al.,* 2006). Epidemiological studies based on ingestion of arsenic have been implicated in noncarcinogenic health effects in various organs and systems including cardiovascular, dermal, reproductive, neurological, respiratory, hepatic, hematological, renal, and gastrointestinal (Tapio, S. *et al.,*

2006). Nonetheless, exposure to arsenic has been associated with the development of malignancies, severe gastrointestinal toxicities, diabetes, cardiovascular diseases, and even death. The relationship of arsenic and malignancy is a major concern. It has been documented that long-term arsenic exposure is associated with increased risks of numerous human cancers (Tapio, S. *et al.,* 2006, and Ghosh, P. *et al.,* 2008), and as a result, arsenic has been classified as a human carcinogen by both the U.S. Environmental Protection

Agency (Tchounwou, PB. *et al*., 2003) and the IARC (IARC, 1987). Exposure to arsenic orally or by inhalation can lead to increased risk of skin and lung cancer respectively (Tchounwou, PB. *et al*., 2003). In addition to lung cancer, cancers of the liver, kidney, lung, colon and bladder are affected with increased arsenic exposure (Tchounwou, PB. *et al*., 2003, Tchounwou, PB. *et al*., 2003, and Tchounwou, PB. *et al*., 1999). The mechanism of action of arsenic in carcinogenesis has not been clearly elucidated (Ghosh, P. *et al.,* 2008, and Tchounwou, PB. *et al*., 2003). Many mechanisms have been implicated for arsenic-induced carcinogenesis, such as genotoxicity, induction of oxidative stress and DNA damage, inhibition of DNA repair enzymes involved in nucleotide excision repair and base excision repair, tumor promotion, cell proliferation, chromosomal aberrations, and signal transduction or altered DNA methylation (Roy, P. *et al*., 2002 and Galanis, A. *et al.,* 2009).

Arsenic toxicity has become a global public health concern and has affected human health. Numerous epidemiological studies have reported that large populations in the world are being exposed chronically to arsenic (Tchounwou, PB. *et al*., 2003). Exposure to inorganic arsenic occurs via environmental (e.g., contaminated drinking water, air, food, domestic fuel sources) and occupational exposures (e.g., smelting industries, pesticide production). The toxicity of arsenic is very complicated and the toxicity varies on its oxidative state and solubility

(Tchounwou, PB. *et al*., 2003, Hughes MF. 2002, and Miller WH. *et al.,* 2002). The trivalent compounds such as arsenic trioxide, sodium arsenite and arsenite, and arsenic trichloride are more toxic than the pentavalent compounds such as arsenic pentoxide, arsenic acid, lead and calcium arsenates (Tchounwou, PB. *et al*., 2003, and Hughes MF. 2002). The trivalent and pentavalent forms of arsenic are found in arsenic-contaminated water (Hughes MF. 2002, and Frumkin, H. *et al*., 2001). This metalloid affects a variety of tissues and cells depending on the degree of exposure. Acute or chronic exposure has been reported in several countries around the world due to the high concentrations found in drinking water (Tchounwou, PB. *et al*., 2004, Guo, H. 2007, and NRC. 2007). The increased cancer risk posed by contaminated water is thought to be due to the presence of inorganic trivalent arsenite (Klein, CB. 2007) The impairment of cellular respiration by the inhibition of various mitochondrial enzymes, and the uncoupling of oxidative phosphorylation is believed to be one of the mechanisms by which arsenic exerts is toxic effect (Tchounwou, PB. *et al*., 2003). Arsenic toxicity is thought to result from its ability to interact with sulfhydryl groups of proteins and enzymes, and to substitute phosphoruous in a variety of biochemical reactions (Tchounwou, PB. *et al*., 2003, and Miller WH. *et al.,* 2002). Research has shown that the major metabolic pathway for inorganic arsenic in humans is methylation in which most of the inorganic arsenic is metabolized to monomethylarsonic acid and dimethylarsinic acid before excretion in the urine (Tchounwou, PB. *et al*., 2003). There is still much discussion as to whether arsenic is a carcinogen by itself or acts synergistically to predispose cells to transformation by complete carcinogens (Kitchin, KT. 2001).

2.3 ORGANS OF STUDY: LARGE INTESTINES

The large intestine is the portion of the digestive tract where water is absorbed from indigestible material. This section of the gut includes the cecum, appendix, entire colon, rectum, and anal canal. The large intestine begins at the terminal ileum with the cecum. Unlike the small intestine, the large bowel is shorter but has a much larger lumen. This segment is further distinguished from the small intestine by the presence of omental appendices, haustra, and teniae coli. (Chaudhry SR. *et al*., 2024, Dumont F. *et al*., 2013, and Smereczyński A. *et al*., 2018) The omental appendices are small pouches of peritoneum filled with fat. The haustra are small pouches or sacculations that give the large intestine its segmented appearance. The teniae coli are 3 longitudinal bands of smooth muscle on the outer wall of the colon.

2.3.1 APPEARANCE AND LOCATION OF THE LARGE INTESTINE

The large intestine, or colon, possesses several distinct external features that make it readily identifiable. Its most characteristic aspects are three specialized anatomical structures. Externally, the colon is marked by the presence of **teniae coli** three distinct, longitudinal bands of smooth muscle that run along its length. Because these bands are shorter than the intestine itself, they cause the colon wall to pucker into a series of sacculations known as **haustra**, giving it a segmented, pouch-like appearance **(Standring, 2021)**. Furthermore, small, fat-filled pouches of the peritoneum called **omental appendices** (or epiploic appendages) are attached to its external surface along the teniae coli **(Drake *et al.,* 2019)**. Collectively, these features the teniae coli, haustra, and omental appendices distinguish the large intestine from all other parts of the gastrointestinal tract.

The large intestine is situated in the abdominal and pelvic cavities, where it describes a characteristic "horseshoe" or frame-like path around the periphery of the abdominal cavity, encircling the coiled mass of the small intestine **(Standring, 2021)**. Its journey begins in the **right lower quadrant** of the abdomen at the **ileocecal junction**. Here, the terminal part of the small intestine, the ileum, opens into the first part of the large intestine, the cecum, through the ileocecal valve, a structure that regulates flow and prevents reflux **(Drake *et al*., 2019)**.

2.3.2 STRUCTURE AND FUNCTION OF THE INTESTINE

The large intestine measures approximately 1.5 meters in length and is divided into several distinct regions: the cecum, colon (ascending, transverse, descending, and sigmoid), rectum, and anal canal (Standring, 2021). The inner wall of the large intestine lacks the villi characteristic of the small intestine but contains numerous goblet cells that secrete mucus to facilitate the smooth passage of fecal material (Young *et al*., 2020). The colon's wall consists of four layers: mucosa, submucosa, muscularis externa, and serosa. The muscularis externa is characterized by three longitudinal muscle bands known as the *teniae coli*, which create pouch-like segments called *haustra* that assist in the movement and mixing of intestinal contents (Hall & Hall, 2020).

The mucosa of the large intestine contains simple columnar epithelium with abundant goblet cells that secrete mucus for lubrication (Mescher, 2018). The epithelium also houses absorptive cells responsible for the uptake of water and electrolytes. Lymphoid tissue is present throughout the submucosa and mucosa, contributing to the gut-associated lymphoid tissue (GALT), which provides immune defense against pathogens (Young *et al*., 2020).

The primary function of the large intestine is to absorb water and electrolytes from indigestible food residues, thereby transforming liquid chyme into semisolid feces. Approximately 1.5 liters of chyme enter the colon daily, of which about 90% of the water is absorbed (Hall & Hall, 2020). This process is facilitated by active sodium transport and passive water movement through osmosis. The colon also absorbs certain vitamins, such as vitamin K and some B vitamins, produced by commensal bacteria residing within the intestinal microbiota (Tremaroli & Bäckhed, 2012).

Bacterial fermentation of undigested carbohydrates in the colon produces short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, which serve as an energy source for colonic epithelial cells and contribute to maintaining intestinal health (Ríos-Covián *et al.,* 2016). The presence of beneficial bacteria in the large intestine also inhibits the growth of pathogenic microorganisms, supporting immune function and gut homeostasis (Cummings & Macfarlane, 1997).

Movement of material through the large intestine occurs via peristaltic and segmental contractions. Periodic mass movements, often occurring a few times daily, propel fecal matter toward the rectum (Hall & Hall, 2020). Defecation is regulated by both involuntary (internal anal sphincter) and voluntary (external anal sphincter) control mechanisms coordinated by the central and enteric nervous systems.

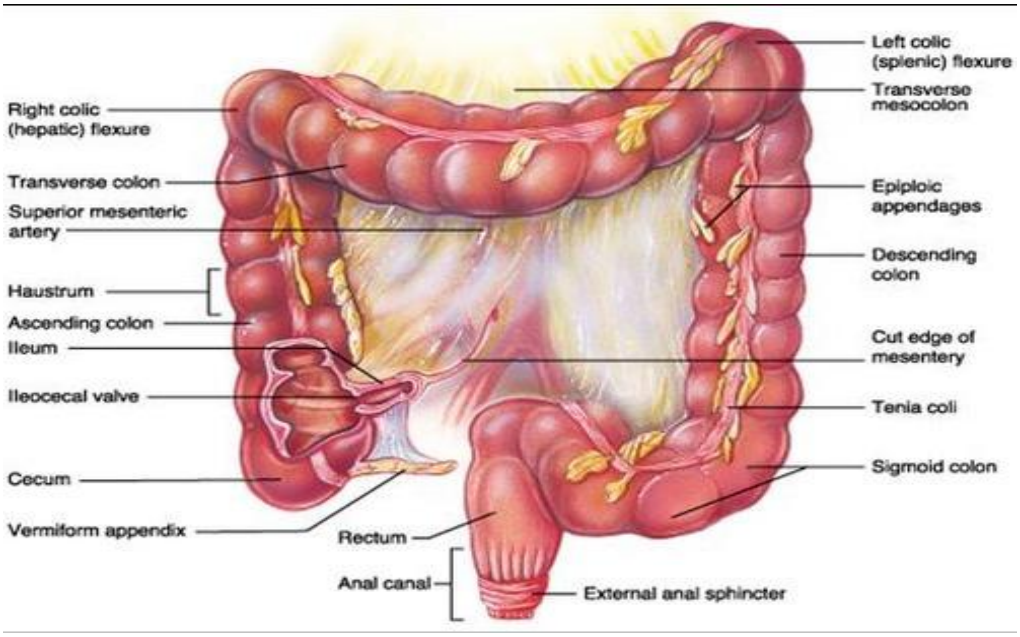

Figure 4: large intestine (source:quizlet.com/344872141/the-large-intestine-labeling-diagram/)

2.3.3 BLOOD SUPPLY

The superior mesenteric artery (SMA) and inferior mesenteric artery (IMA) supply blood to the large intestine. These 2 vessels communicate through the marginal artery, which runs parallel to the entire length of the colon. The cecum receives blood from the ileocolic artery, a terminal branch of the SMA. This artery also gives rise to the appendicular artery, which supplies the appendix. The ascending colon and right colic flexure derive their blood supply from the ileocolic and right colic arteries, both branches of the SMA. The transverse colon primarily receives arterial supply from the middle colic artery, another branch of the SMA. Additional blood flow comes from anastomotic arcades between the middle and left colic arteries, which contribute to the formation of the marginal artery (of Drummond). The descending and sigmoid colonic segments are perfused by the left colic and sigmoid arteries, branches of the

IMA. The transition of blood supply at the left colic flexure, where the SMA gives way to the IMA, marks the embryological shift from the midgut to the hindgut. The rectum and anal canal receive blood from multiple sources. The superior rectal artery, a continuation of the IMA, supplies the upper portion. Additional contributions come from the middle rectal artery, a branch of the internal iliac artery, and the inferior rectal artery, which arises from the internal pudendal artery. (Bruzzi M. *et al*., 2020, and Griffiths JD. 1956).

Venous drainage generally follows the arterial supply. The inferior mesenteric vein (IMV) drains into the splenic vein, while the superior mesenteric vein (SMV) joins the splenic vein to form the hepatic portal vein. Lymphatic drainage of the large intestine follows the pathways of the primary blood vessels, directing fluid to their associated lymph nodes. (Harkins JM. 2023).

2.3.4 NERVE SUPPLY

The large intestine possesses its own intrinsic nervous system, the enteric nervous system (ENS), which is capable of mediating local reflexes independently of the central nervous system (Furness, 2012). The ENS within the colonic wall consists of two primary plexuses. The myenteric plexus (Auerbach's plexus), located between the longitudinal and circular muscle layers, is the principal regulator of colonic motility. It controls the haustral churning that facilitates water absorption and generates the powerful peristaltic waves known as mass movements (Tortora & Derrickson, 2017). The submucosal plexus (Meissner's plexus), situated in the submucosa, primarily influences secretory activity and local blood flow, fine-tuning the fluid environment within the colon (Sherwood, 2016).

The extrinsic autonomic innervation provides overarching modulation of the ENS and connects colonic function to the central nervous system. The autonomic supply to the proximal

colon (up to the distal transverse colon) originates from the superior mesenteric plexus and is supplied by the vagus nerve (CN X), representing its parasympathetic "rest-and-digest" influence (Standring, 2016). Vagal stimulation enhances propulsive motility and secretory functions in this region. In contrast, the parasympathetic supply to the distal colon (from the splenic flexure to the upper rectum) is provided by pelvic splanchnic nerves (S2-S4), which are distinct from the vagus (Drake, Vogl, & Mitchell, 2019). These nerves are crucial for initiating the defecation reflex, stimulating mass movement, and relaxing the internal anal sphincter.

The sympathetic innervation to the entire large intestine arises from the lower thoracic and lumbar spinal cord (T10-L2). Preganglionic fibers synapse in prevertebral ganglia—the superior mesenteric ganglion (for proximal colon), inferior mesenteric ganglion (for distal colon), and the hypogastric plexus (for the rectum) before their postganglionic fibers travel along arterial pathways to the gut wall (Moore, Dalley, & Agur, 2018). Sympathetic activity is universally inhibitory, acting to suppress colonic motility, inhibit secretion, and cause contraction of the internal anal sphincter. This represents the "fight-or-flight" response, halting non-essential digestive processes during stress (Sherwood, 2016). The innervation of the rectum and anal canal demonstrates a unique transition from autonomic to somatic control, which is essential for maintaining continence. The smooth muscle of the internal anal sphincter is under involuntary control via sympathetic (excitatory, causing contraction) and parasympathetic pelvic splanchnic nerves (inhibitory, causing relaxation) (Standring, 2016). In stark contrast, the external anal sphincter, composed of skeletal muscle, is under voluntary somatic motor control supplied by the inferior rectal nerve, a branch of the pudendal nerve (S2-S4) (Drake *et al.,* 2019). This dual control allows for the conscious decision to delay defecation until it is socially appropriate.

Sensory innervation is equally critical. Stretch receptors in the rectal wall detect distension by fecal matter, transmitting this sensation via pelvic splanchnic nerves to trigger the urge to defecate. Furthermore, the anal canal possesses specialized sensory nerve endings that can discriminate between solid, liquid, and gaseous contents, a function vital for fine continence (Furness, 2012). Damage to the pelvic splanchnic nerves or the sacral spinal cord can lead to fecal incontinence or severe constipation. Conversely, the failure of the enteric neural crest cells to migrate (Hirschsprung's disease) results in a functional obstruction in the distal colon due to the absence of ganglion cells in the ENS (Moore *et al.,* 2018).

2.3.5 LYMPHATIC DRAINAGE

The lymphatic drainage of the large intestine is arranged in a hierarchical network that begins within the intestinal wall and extends to regional lymph nodes. The mucosa and submucosa of the large intestine contain an extensive array of lymphatic capillaries and lymphoid aggregates collectively known as *gut-associated lymphoid tissue* (GALT) (Standring, 2021). These lymphatic capillaries are particularly abundant in the lamina propria, where they collect lymph and transport immune cells and antigens toward larger lymphatic vessels.

The GALT of the large intestine includes isolated lymphoid follicles and, occasionally, aggregated lymphoid nodules similar to Peyer’s patches found in the small intestine (Mescher, 2018). These follicles are composed primarily of B lymphocytes, T lymphocytes, macrophages, and antigen-presenting cells, which together coordinate immune responses against microbial antigens (Young *et al*., 2020). Lymph from the intestinal wall drains sequentially into epicolic, paracolic, intermediate, and main colic lymph nodes, ultimately converging at the superior and

inferior mesenteric lymph nodes before entering the cisterna chyli and thoracic duct (Hall & Hall, 2020).

2.3.6 EMBRYOLOGY

The large intestine has a dual embryonic origin. The cecum, appendix, ascending colon, and the proximal two-thirds of the transverse colon are derived from the **midgut**, which is supplied by the **Superior Mesenteric Artery (SMA)**. The distal third of the transverse colon, the descending colon, sigmoid colon, rectum, and upper anal canal are derived from the **hindgut**, which is supplied by the **Inferior Mesenteric Artery (IMA)** (Sadler, 2019). The cloaca, an endodermal-lined cavity, gives rise to the rectum and upper anal canal, while the lower anal canal originates from the surface ectoderm of the **proctodeum** (Moore, Persaud, & Torchia, 2016).

The most critical event in the development of the midgut portion is the **physiological umbilical herniation**. Around the sixth week of development, the rapidly elongating midgut loop, coupled with the expansive growth of the liver, creates a space deficit within the abdominal cavity. This forces the midgut loop to herniate into the umbilical cord (Larsen, 2001). This is a normal and necessary event. While herniated, the loop rotates **90 degrees counterclockwise** around the axis of the SMA. This initial rotation positions the pre-arterial limb (future small intestine) to the right and the post-arterial limb (which contains the primitive cecal bud) to the left.

Around the tenth week, the abdominal cavity has enlarged, and the midgut retracts back into the abdomen in a specific sequence. The small intestine returns first, filling the central area. As the returning loop undergoes a further **180-degree counterclockwise rotation**, bringing the

total rotation to **270 degrees**, the cecum and appendix are the last structures to re-enter the abdominal cavity (Standring, 2016). Initially, the cecal bud is positioned high in the right upper quadrant, just below the liver. From this position, it **descends** to its final adult location in the right iliac fossa, giving rise to the ascending colon and appendix (Sadler, 2019).

Concurrently, the hindgut undergoes its own development. The **cloaca**, the distal end of the hindgut, is partitioned by the **urorectal septum** into a dorsal **anorectal canal** (future rectum and upper anal canal) and a ventral **urogenital sinus** (which will form bladder and urethral structures) (Moore *et al*., 2016). The point of fusion of the urorectal septum with the cloacal membrane becomes the **perineal body**. The endodermal epithelium of the upper anal canal derives from the hindgut, while the lower anal canal originates from an invagination of surface ectoderm called the **proctodeum**. The junction between these two embryological regions is marked by the **pectinate line**, a critical landmark with distinct anatomical, histological, and neurovascular characteristics above and below it (Tortora & Derrickson, 2017).

Following rotation, the process of **fixation** occurs. The mesenteries of the gut fuse with the posterior parietal peritoneum. The ascending and descending colon, which are retroperitoneal structures, become fixed to the posterior abdominal wall through this process of **retroperitonealization** (Larsen, 2001). The transverse colon and sigmoid colon retain their mesenteries (transverse mesocolon and sigmoid mesocolon) and remain intraperitoneal.

. **Malrotation** occurs when the normal 270-degree counterclockwise rotation fails, leaving the cecum and appendix improperly positioned and creating a risk for volvulus (Moore *et al*., 2016). Failure of the cecum to descend can result in a **subhepatic cecum and appendix. Imperforate anus** results from a failure of the anal membrane to perforate,

while **Hirschsprung's disease** (congenital aganglionic megacolon) is caused by the failure of neural crest cells to migrate into the hindgut wall, resulting in a functional obstruction (Sadler, 2019).

2.3.7 HISTOLOGY

The innermost layer, the **mucosa**, is architecturally distinct from that of the small intestine. Most notably, it lacks **villi** and **circular folds (plicae circulares)**, resulting in a relatively smooth surface area. However, its absorptive capacity is maintained through deep, tubular invaginations known as **intestinal glands (crypts of Lieberkühn)** (Ross & Pawlina, 2016). The surface epithelium and the linings of these glands are simple columnar epithelium, populated predominantly by two key cell types: **absorptive cells** and **goblet cells**. The absorptive cells possess microvilli on their apical surface to amplify the area for water and electrolyte absorption (Mescher, 2021). **Goblet cells** are far more numerous in the large intestine than in the small intestine. They continuously secrete mucus to lubricate the intestinal lining, facilitating the passage of fecal material and protecting the mucosa from abrasion and from the resident bacterial flora (Kierszenbaum & Tres, 2016). The **lamina propria** is rich in lymphatic tissue, including solitary lymphatic nodules that frequently extend into the submucosa, forming a crucial part of the gut-associated lymphoid tissue (GALT) for immune surveillance. The **muscularis mucosae** remains a thin layer of smooth muscle separating the mucosa from the submucosa. The **submucosa** is a dense connective tissue layer containing the larger branches of blood vessels, lymphatic vessels, and nerves. A key feature here is the **submucosal (Meissner's) plexus**, which is part of the enteric nervous system and regulates secretory activity in the mucosal glands (Tortora & Derrickson, 2017). Unlike the duodenum, the large intestine does not

contain Brunner's glands in its submucosa. The **muscularis externa** of the large intestine is unique in its organization. It consists of the standard inner circular and outer longitudinal layers of smooth muscle. However, the outer longitudinal layer is not uniformly thick around the entire circumference. Instead, it is gathered into three distinct, equidistant, thick bands called **taeniae coli** (Standring, 2016). The contraction of these bands, which are shorter than the underlying circular muscle, gathers the colon into a series of pouches called **haustra**, giving the colon its characteristic sacculated appearance. This arrangement is efficient for the haustral churning that mixes the contents to maximize water absorption. Between the two muscle layers lies the **myenteric (Auerbach's) plexus**, which controls the motility of the muscularis externa (Sherwood, 2016). The outermost layer varies by anatomical location. Most of the large intestine, being intraperitoneal or retroperitoneal, is covered by a **serosa**, which is a thin layer of connective tissue and mesothelium. In some retroperitoneal areas, such as the ascending and descending colon, the posterior surface is covered by an **adventitia**, which is a connective tissue layer that blends with the surrounding retroperitoneal tissues (Mescher, 2021). The histology of the **anal canal** marks a significant transition. The simple columnar epithelium of the rectum abruptly changes at the **pectinate line** to non-keratinized stratified squamous epithelium. This change is a critical adaptation for withstanding the abrasion associated with the passage of solid feces (Ross & Pawlina, 2016). The muscularis externa of the rectum thickens to form the **internal anal sphincter** (involuntary, smooth muscle). Just distal to this, the circular skeletal muscle of the external anal sphincter (voluntary) appears, underscoring the dual control of defecation.

CHAPTER THREE

MATERIAL AND METHODS

3.1 PLANT COLLECTION AND IDENTIFICATION

The tiger nut was purchased at uselu market in Egor local Government Area of Edo state Nigeria. Thereafter voucher number and identification were carried out in the voucher and identification unit of department of plant Biology and Biotechnology (PBB) in university of Benin where it was identified as Cyperus esculentus.the nuts were thoroughly washed to remove dirt and debris under running water.it was dried for about 2 weeks and pulverised into powdered form using electric grinding machine. After grinding into powdered form, it was soaked with distilled water at the ratio of 1gram to 10ml of the distilled water. It was soaked for about 24 hours with constant stirring. After 24hrs of soaking it was filtered and the residues were discarded and the filterates were concentrated using water baths and preserved in the refrigerator.

3.2 INSTRUMENTATION/EQUIPMENT AND REAGENT

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

- Plastic Cages were the animals were housed.
- Ceramic and plastic plates for placement of feed and water for the rats.
- Electric scale for weighing the extracts and animals.
- Dissecting set for sacrificing the animals.
- Syringes for obtaining blood from the animals and administering extracts and to them.
- Measuring glass cylinder for preparing the extract.
- Plain and luteum heparin bottles for storage of blood.
- Disposable gloves for protecting one's self.

- Microtome, slide tray, tissue embedding stations all for tissue processing.
- Microscope for examining the tissue slide.

Some of the reagents used throughout the duration of this include: Distilled water, Ascending and descending grades of alcohol, paraffin wax, Xylene, Haematoxylin, Eosin, Chloroform, Normal saline, 10% formal saline.

3.3 PREPARATION OF EXTRACT

Once soaked and softened, the tiger nuts are rinsed and transferred to a **blender**. Clean water is added, usually at a ratio of about **2 cups of tiger nuts to 3 or 4 cups of water**, depending on the desired consistency of the extract. At this stage, **optional flavoring ingredients** may also be introduced. Common additives include **pitted dates** for additional sweetness, **fresh ginger** for a spicy kick, and **vanilla extract** or **cinnamon** for enhanced aroma and taste. These flavorings not only improve palatability but may also contribute their own health benefits. The mixture is blended until a smooth consistency is achieved. This step is crucial, as it helps to break down the tiger nuts and release their nutrients into the liquid. Once blended, the next step is to **strain the mixture** to separate the milk from the fibrous residue. This is typically done using a **fine sieve, cheesecloth**, or **muslin cloth**. The liquid extracted during this process constitutes the tiger nut milk, while the remaining pulp rich in fiber can be repurposed for baking, used in smoothies, or discarded responsibly. After straining, the tiger nut extract is poured into a clean, airtight **bottle or container** and stored in a **refrigerator**. The extract is best consumed within **three to four days**, as it is a natural product without preservatives. To extend shelf life slightly, mild **pasteurization** (gentle heating) may be employed, though this can alter the flavor and reduce the raw nutritional content.

3.4 ANIMAL CARE AND MANAGEMENT

Twenty adult Wistar rats weighing between 120g and 216g were used for the experiment. The rats were purchased from the animal house in the department of anatomy, University of Benin, Edo State. The rats were then acclimatized for two weeks (at the animal house in the department of anatomy, University of Benin, Edo State) before the commencement of the study. During this period, the animals were allowed free access to standard animal feed (Top feed growers mash.) and clean water ad libitum. Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

3.5 METHOD OF ADMINISTRATION/CHOICE OF DOSAGE

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

3.6 EXPERIMENTAL DESIGN

Twenty (20) experimental adult Wistar rats of either sex were randomly assigned into four (4) groups. They were fed with standard animal feed and clean water.

Groups A - D comprising of five rats per group.

Group A: Served as control.

Group B: Rats were treated daily with oral administration 10mg/Kg of arsenic trioxide only

Group C: Rats were treated with oral administration of medium dose (200mg/Kg) tigernut extract + 10mg/Kg arsenic trioxide.

Group D: Rats were treated with oral administration of high dose (400mg/Kg) tigernut extract + 10mg/Kg arsenic trioxide

3.7 METHOD OF SACRIFICE AND SAMPLE COLLECTION

At the end of the twenty-eight (28) days treatment, the rats were weighed using a weighing scale. Cotton wool was put into an enclosed container with about 45mls of chloroform as anaesthesia. After anaesthetising the rats for about two minutes, the rats were then placed in a supine position on a dissecting table. An abdominal incision was made to expose the abdominal viscera. The Intestine was harvested and immediately fixed in 10% formal-saline for histological analysis. Blood samples were collected using 5mls syringes from abdominal aorta and heart through arterial and cardiac puncture. The samples were put into heparin bottles for renal function analysis. Both the harvested organ (Intestine) and blood samples were then taken to the Histopathology laboratory of University of Benin Teaching Hospital (UBTH) for tissue processing and functional analysis respectively.

3.8 PARAFFIN TISSUE PROCESSING

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

- Dehydration of tissue in an increasing gradient of 70% to 90% alcohol and absolute alcohol using ethanol as the choice of alcohol.
- Clearance of alcohol was done using xylene as a clearing agent. Tissues were allowed to pass through two changes for total removal of alcohol

- The tissues were infiltrated in three changes of molten paraffin wax in an oven at a temperature of 65-70^{O}C. The changes were done for 15 minutes each, and the last changes of paraffin wax for 30 minutes.
- Embedding was carried out using an embedding mould, into which the molten paraffin wax was poured and the infiltrated tissues were placed in it in a longitudinal orientation to produce longitudinal section.
- The molten paraffin wax was allowed to cool resulting in solidification to form tissue block.
- After trimming, sectioning of the tissue blocks was done using the rotary microtome to cut tissue into thin ribbon like section of thickness of 5 microns.

3.9 HEMATOXYLIN AND EOSIN STAINING METHOD

- Satisfaction and good tissue section which came out as ribbon were selected and placed in 20% alcohol for spreading of the paraffin sections which are then cut and floated in a water bath at a temperature of 30^{o}C.
- The sectioned tissues were picked with slides and allowed to dry.
- The tissue sections were placed in xylene for 15minutes to remove excess paraffin wax from the tissues and were then subjected to hydration by passing them through descending grades of alcohol (100%, 90%, 70%) and then into water, all of which lasted for 5minutes each.
- Staining of the tissue was done using H&E dyes. The tissues were stained in hematoxylin for 10 minutes.
- Tissues were washed in running tap water (a process called bluing)
- Sectioning was counter-stained with 1% Eosin for 5-10minutes.

- Tissues were rinsed in water
- Tissues were dehydrated rapidly through 70% graded alcohol to absolute alcohol for 5minutes.
- Tissues were then finally cleared using xylene for 5 minutes and the slides were mount with glass cover slip using a suitable mountant, Distrene Plasticizer and Xylene (DPX).

3.10 PHOTOMICROGRAPHY

The stained slides were analysed by Dr. G. I Eze, of the department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin. Sections of the tissues were examined under a Leica DM750 research microscope with a digital camera Leica ICC50 attached and connected to a Hewlett-Packard laptop. Digital photomicrographs of the tissue section were taken at various magnifications. The photomicrographs were then analysed.

3.11 STATISTICAL ANALYSIS

The was analyzed using descriptive and inferential statistics. All values were presented as mean standard error of mean (SEM). The significance of difference in the means of all parameters was determined using one-way analysis of variance (ANOVA: 95% confidence interval). Least square difference, post hoc tests were carried out for all groups with control and comparison of all pairs of groups respectively using Bonferoni multiple comparison. All statistical analysis was carried out using Statistical package for Social Sciences (SPSS).

CHAPTER FOUR

RESULT

4.1 RESULTS OF STATISTICAL ANALYSIS

The following results below were obtained after a period of administration of feed and water across the groups of eight different groups of Wister rats.

- Chart 1 showing initial and final body weight within group
- Chart 2 showing weight change across group

Data were subjected to statistical analysis using the IBM SPSS statistics software and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean ≠SEM.LSD post-hoc test was used. Values of $P<0.05$ were considered significant. The Statistical values obtained were converted into tables and bar charts.

4.1.1 CHART 1: BODY WEIGHT

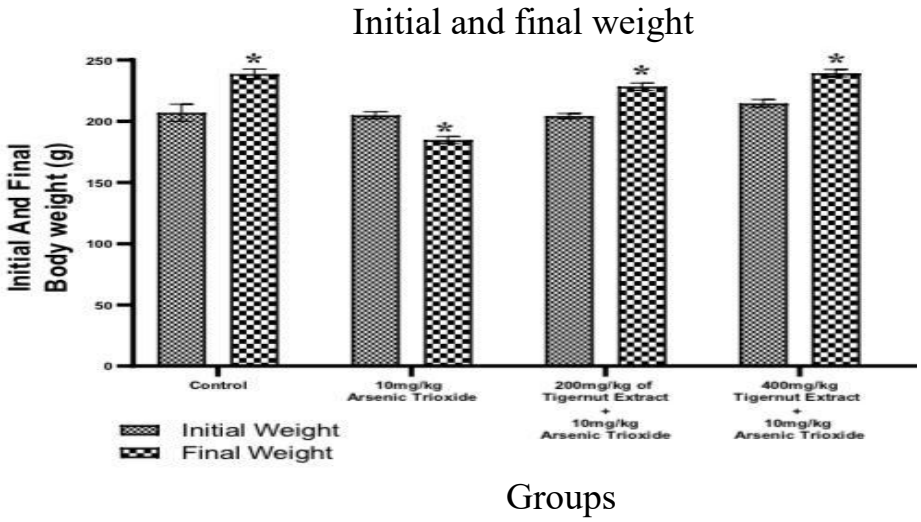

Chart showing the initial weight in comparison to the final body weight.

significantly different from the initial body weight

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

4.1.2 CHART 2: WEIGHT CHANGE ACROSS GROUPS

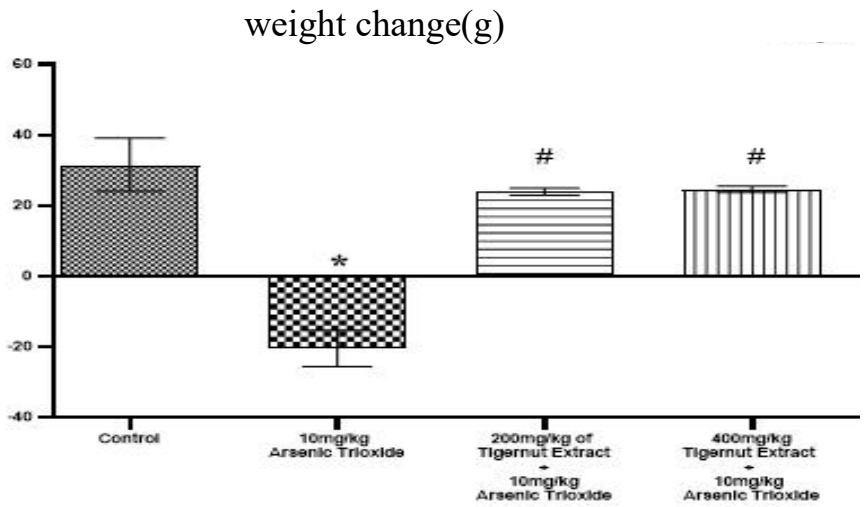

Chart shows the mean weight change for each experimental group.

Significant difference compared to control,

Significant difference compared to Arsenic Trioxide only.

4.2 HISTOLOGY PLATE

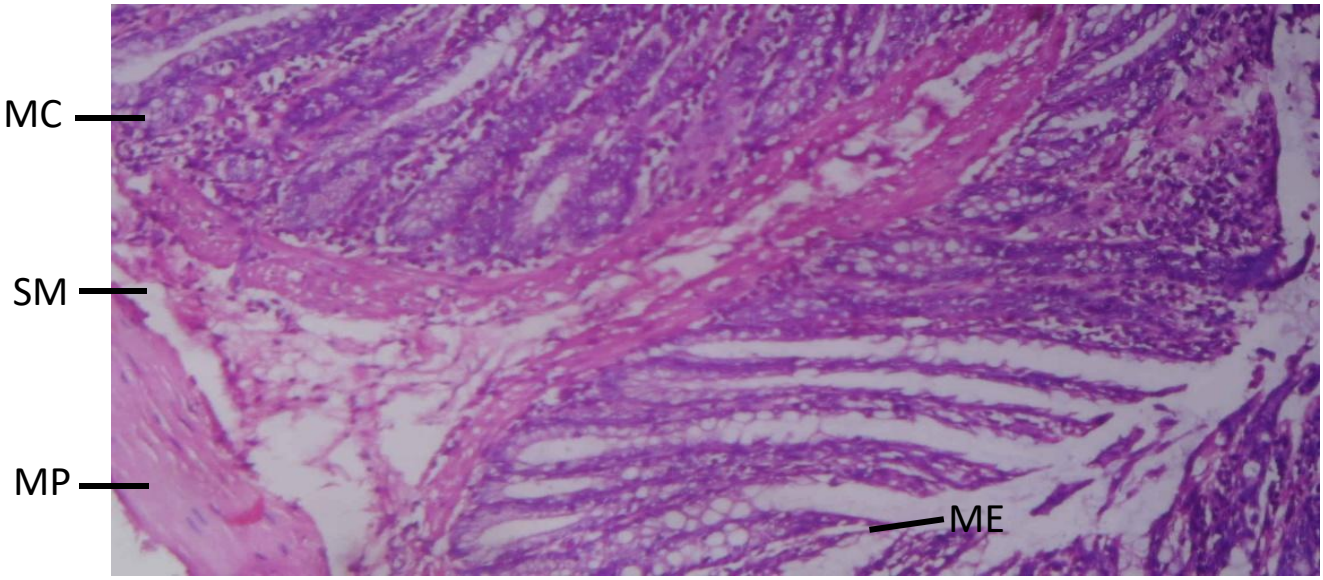

Plate 1. Rat large intestine, control, show normal architecture: mucosal membrane (ME), crypts (MC), submucosa (SM) and muscularis propria (MP): H&E 100 X

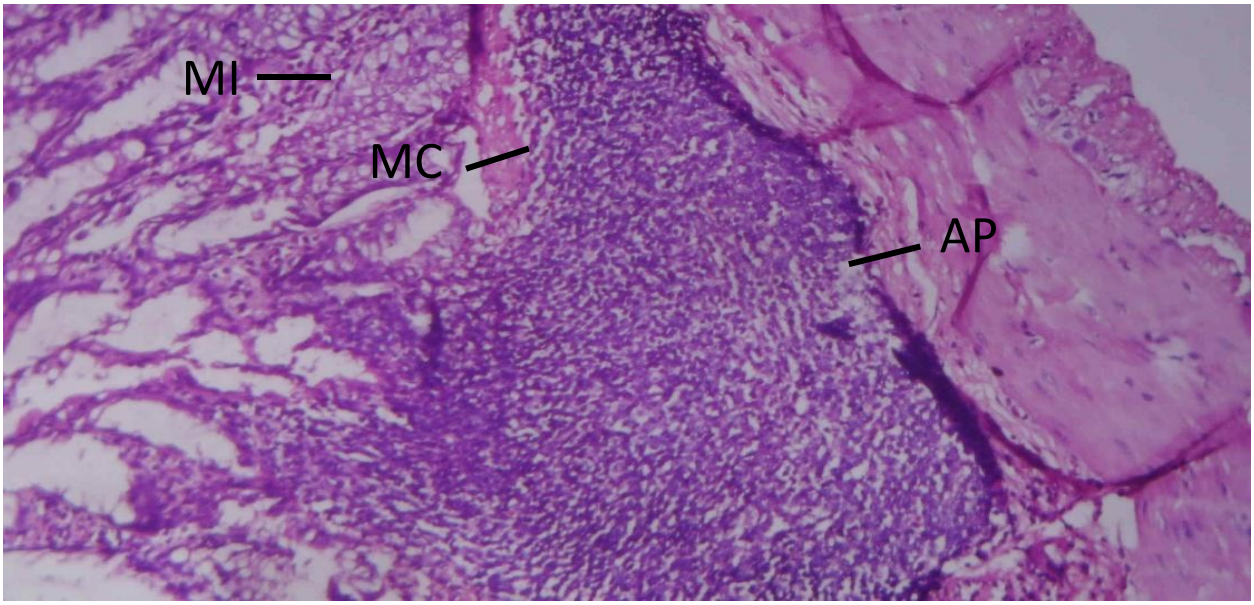

Plate 2. Rat large intestine given Arsenic only show: mural infiltrates of inflammatory cells (MI), activated payers patches (AP) and mural congestion (MC) : H&E 100 X

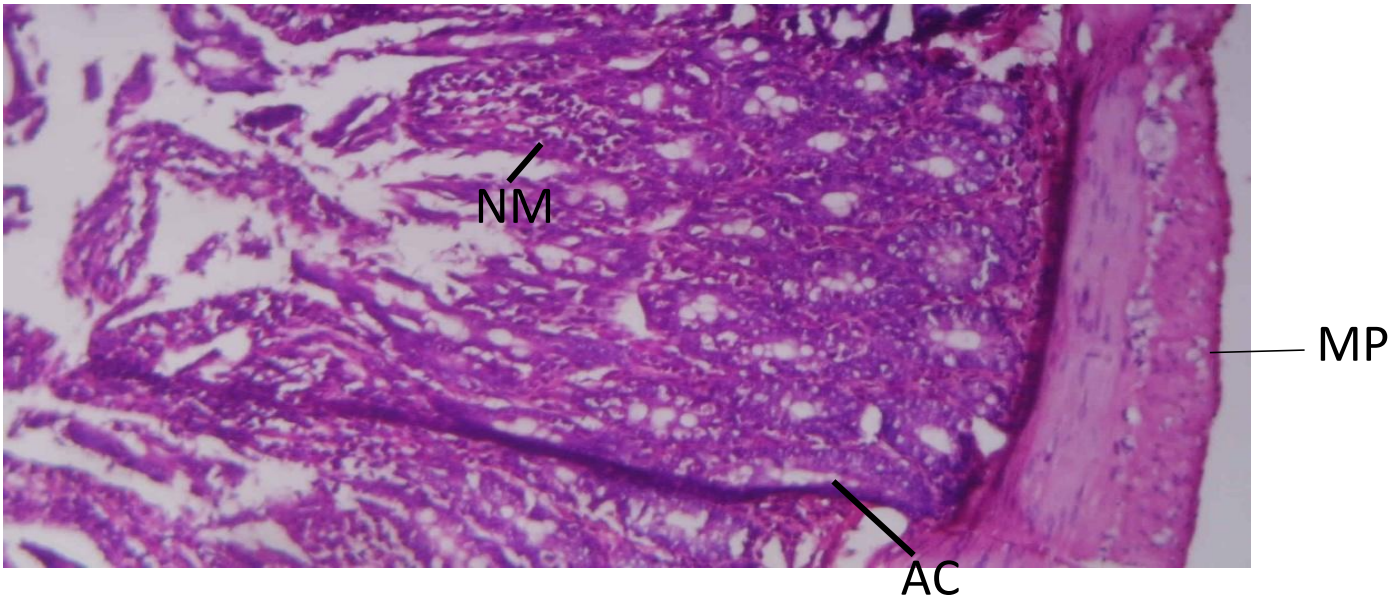

Plate 3. Rat intestine given 200mg Cyperus Esculentus + Arsenic show normal mucosa (NM), active mural congestion (AC) and normal muscularis propria (NP) : H&E 100 X

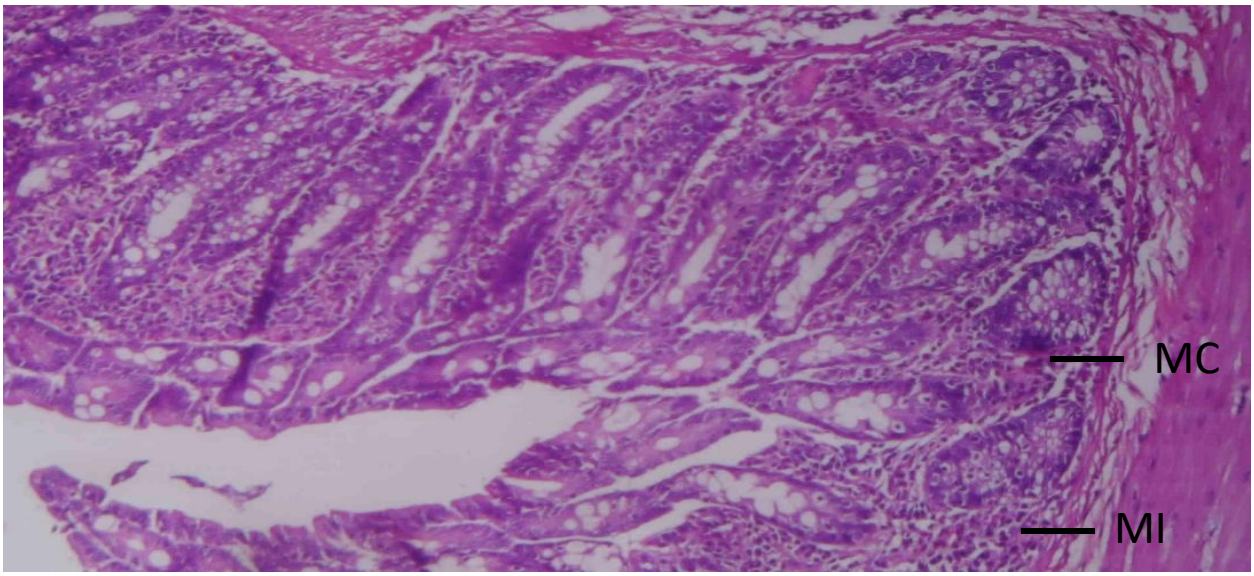

Plate 4. Rat intestine given 400mg Cyperus Esculentus + Arsenic show heavy mucosal infiltrates of inflammatory cells (MI) and mural congestion (MC) : H&E 100 X

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study was to determine the effect of aqueous extract of *Cyperus esculentus* against arsenic trioxide induced damage on the large intestine across group of adult Wistar rats. Arsenic is a well know human toxicant and carcinogenic metalloid. Exposure to arsenic and its compounds can have adverse effects on human health (Tapio, S. *et al.,* 2006). Epidemiological studies based on ingestion of arsenic have been implicated in noncarcinogenic health effects in various organs and systems including cardiovascular, dermal, reproductive, neurological, respiratory, hepatic, hematological, renal, and gastrointestinal (Tapio, S. *et al.,* 2006). Nonetheless, exposure to arsenic has been associated with the development of malignancies, severe gastrointestinal toxicities, diabetes, cardiovascular diseases, and even death. The relationship of arsenic and malignancy is a major concern. It has been documented that long-term arsenic exposure is associated with increased risks of numerous human cancers (Tapio, S. *et al.,* 2006, and Ghosh, P. *et al.,* 2008), and as a result, arsenic has been classified as a human carcinogen by both the U.S. Environmental Protection.

The average weight of the wistar rats in each group at the end of the experiment was compared to the previous initial average weight of the wistar rats in the same group before the experiment. Across the group there was an increase in the final weight of experimental animal when compared to their initial weights except for the one administered with only arsenic trioxide. That is to say arsenic trioxide can hinder the growth in term of weight gain. Individuals exposed

to arsenic trioxide may experience a range of gastrointestinal symptoms, including diarrhea, abdominal pain, and impaired nutrient absorption (Shakoori et al., 2022).

On histological analysis, Sections treated with Arsenic only shown to cause mural infiltrates of inflammatory cells and mural congestion. Sections of the large intestine taken from wistar rats given baseline feeds and water randomly (normal control), and rats treated with 200mg/kg body weight tigernut extract + Arsenic trioxide, all show normal histological architecture, with well-defined mucosa, submucosa, muscularis propria and serosa, including their constituent parenchymal cells, Brunner's glands, and connective tissue support. Sections treated with 400mg/kg body weight tigernut extract and Arsenic trioxide, show normal intestinal wall with lingering infiltrates of inflammatory cells. This implies that protection was not complete. Thus, when tigernut extract was administered in a protective protocol with Arsenic trioxide, there is good protection against the inflammation caused by Arsenic trioxide. The antioxidants in the tigernut help neutralize harmful free radicals in the body, reducing oxidative stress, which is a key contributor to chronic diseases, aging, and inflammation **(Codina-Torrella *et al*., 2015)**.

5.2 CONCLUSION

In conclusion, the findings from this study provide compelling evidence that the aqueous extract of *Cyperus esculentus* possesses a significant protective effect against arsenic trioxide-induced intestinal damage in Wistar rats.

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