EFFECT OF AQUEOUS-METHANOL EXTRACT OF THE ROOTS OF *Azanza garckeana* ON HISTOLOGICAL PARAMETERS OF WISTAR ALBINO RATS

BY

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CERTIFICATION

We the undersigned, certify that **ALONGE PRAISE EMMANUEL** with matriculation number **LSC1806254** carried out this project work in partial fulfillment of the requirements for the award of Bachelor of Science (**B.Sc**, Hons) Degree in Biochemistry, in the Department of Biochemistry.

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EXTERNAL EXAMINER DATE.

DEDICATION

To my beloved parents, who have been my source of inspiration and strength against the thought of giving up, to whom I attribute all my efforts and struggles of the educational life, who continually provide their all-round support.

To God Almighty, my Daddy, the author of true knowledge and wisdom, without whom I'm naught.

ACKNOWLEDGEMENT

The completion of this undertaking could not have been possible without the participation and assistance of so many people whose names may not all be enumerated. Their contributions are sincerely appreciated and gratefully acknowledged. However, this writer would like to express his deep appreciation and indebtedness particularly to the following:

I wish to register my profound gratitude to the Almighty God for his mercy and grace in my life nonpareil. Also, I deem it fit to extend special regards to my wonderful parents extraordinaire, Pst. & Pst. (Mrs) E. B. Alonge, who are the source of any success in my life. I wish to extend my sincere gratitude to my project supervisor, Professor (Mrs.) B. O. Agoreyo, Department of Biochemistry for her guidance and constructive criticism which has been absolutely helpful in the successful completion of this undertaking. I am indebted to Prof. Onyeneke, Professor and Head, Department of Biochemistry for his facilitative support. The Department of Biochemistry, University of Benin for her facilitative grace. To my super-sub supervisor, Ms. Ofure A. (*MSc in view*).

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ABSTRACT

The study investigates the impact of the aqueous-methanol extract of Azanza garckeana on histological parameters in Wistar albino rats. Azanza garckeana, commonly known as African wild loquat, is recognized for its medicinal properties. The project aims to explore its potential effects on the microscopic structure of tissues in rats, focusing on organs such as the liver, kidney, and heart. The research methodology involves the extraction of bioactive compounds from *Azanza garckeana* using a mixture of water and methanol, followed by administration to the experimental group of rats. Histological analyses are conducted to assess any alterations in tissue architecture, cellular morphology, and pathological changes compared to the control group. Histopathological findings from this study revealed no alteration in the organs; liver, kidney, heart, colon and testis of male Wistar albino rats administered aqueous-methanol root extract of Azanza garckeana. These findings indicate that the plant extract did not compromise the cellular integrity nor inflict damage to these organs. The findings provide insights into the potential therapeutic or adverse effects of Azanza garckeana extract on organ health, contributing to our understanding of its pharmacological properties. This investigation holds significance in the field of biochemistry by elucidating the biological mechanisms underlying the effects of natural products on histological parameters, which could pave the way for further exploration of *Azanza garckeana* as a potential source of novel pharmaceutical agents.

CHAPTER ONE

INTRODUCTION

Throughout the world, many cultures have employed the utilization of natural remedies for the treatment of diseases. (Elufioye and Onoja, 2015). According to the World Health Organization (2018), about 80% of the global population in developing countries depends on natural products for basic and primary healthcare needs. Furthermore, virtually all human diseases including infectious disease, inflammatory, analgesic, diabetics, malaria, hepatorenal diseases and disease associated with oxidative stress have been treated or attenuated with medicinal plants (Umar *et. al*., 2019)

1.1 Azanza Garckeana

Azanza garckeana, commonly known as "wild custard apple" or "monkey bread," is a plant native to Africa, particularly found in countries like Nigeria, Senegal, Sudan, and Ethiopia. Belonging to the Malvaceae family, it's a small tree or shrub characterized by its greenish-yellow, round fruit. (Orwa *et al*., 2009). In Nigeria specifically, it is native to the northeastern region, particularly in the Tula Village of Gombe state where it is used locally as a source of nutrition and medication. (Ochokwu *et al.*, 2019). It is commonly referred to as Goron Tula in the Hausa language.

1.1.1 Taxonomical and Botanical Description

The taxonomical classification of *Azanza garckeana* is as follows:

- Kingdom: Plantae (Plants)

- Clade: Tracheophytes (Vascular plants)

- Clade: Angiosperms (Flowering plants)

- Clade: Eudicots

- Clade: Rosids

- Order: Malvales

- Family: Malvaceae (Mallow family)

- Genus: Azanza

- Species: *Azanza garckeana*,

This classification places it within the broader context of plant taxonomy, specifically within the order Malvales and the family Malvaceae, which includes other plants like cotton, okra, and hibiscus.

The botanical description of *Azanza garckeana* typically includes the following characteristics:

Habit: *Azanza garckeana* is a small tree or shrub that can reach heights of up to 6 meters (20 feet). It has a bushy and often spreading growth habit.

Leaves: The leaves are alternate, simple, and palmately lobed, typically with 3 to 5 lobes. They are dark green in color and have a slightly rough texture.

Flowers: The flowers of *Azanza garckeana* are small and inconspicuous, usually greenish-yellow to yellow in color. They are borne in clusters and have five petals.

Fruit: The fruit of *Azanza garckeana* is a round or oval drupe, typically greenish-yellow when ripe. It is fleshy and contains numerous small seeds embedded in pulp.

Bark: The bark of *Azanza garckeana* is rough and grayish-brown in color, with fissures and ridges as the plant matures.

Roots: The roots of *Azanza garckeana* are shallow and fibrous, aiding in anchoring the plant in the soil.

These botanical characteristics provide a visual and structural overview of *Azanza garckeana*, aiding in its identification and classification in its natural habitat. (Mojeremane and Tshwenyane, 2004).

1.1.2 Distribution

Azanza garckeana is widely distributed across various regions of Africa, particularly in the sub-Saharan belt. Specific countries and regions where it is commonly found include:

West Africa: *Azanza garckeana* is prevalent in countries such as Nigeria, Senegal, Ghana, Mali, Burkina Faso, and Niger. It thrives in both savanna and woodland habitats in this region. (Ochokwu *et al.,* 2015)

East Africa: The plant is also found in countries like Ethiopia, Sudan, Kenya, Tanzania, and Uganda. It is often encountered in semi-arid and arid areas, as well as along riverbanks and in forest clearings.

Central Africa: *Azanza garckeana* occurs in countries such as Cameroon, Central African Republic, Democratic Republic of the Congo, and Republic of the Congo. It is commonly found in both humid and semi-humid forest habitats in this region.

Southern Africa: While less common in southern Africa compared to other regions, *Azanza garckeana* can still be found in countries like Angola, Zambia, Zimbabwe, and Botswana. It is typically found in savanna and woodland ecosystems in this part of the continent.

Overall, the geographical distribution of *Azanza garckeana* spans a wide range of ecological zones across Africa, reflecting its adaptability to diverse habitats. It is often encountered in areas with a dry or semi-arid climate, as well as in forest clearings, riverbanks, and savanna woodlands (Mojeremane and Tshwenyane, 2004).

1.1.3. Historical And Cultural Significance Of *Azanza garckeana* In African Culture

The historical and cultural significance of *Azanza garckeana* in African traditional medicine and culinary practices spans centuries, rooted in indigenous knowledge and cultural heritage.

Traditional Medicine: *Azanza garckeana* has been an integral part of African traditional medicine for generations, with various parts of the plant used to treat a wide range of ailments. The bark, roots, leaves, and fruit of *Azanza garckeana* are all utilized in traditional medicinal preparations. It is believed to possess diverse medicinal properties, including anti-inflammatory, antimicrobial, antidiabetic, and antimalarial effects. In different regions, it has been used to treat gastrointestinal disorders, respiratory ailments, skin conditions, fevers, and malaria, among other health issues. Traditional healers and herbalists often prepare decoctions, infusions, poultices, or extracts from *Azanza garckeana* for medicinal use. (Nkafamiya *et al*., 2015)

Culinary Practices: The fruit of *Azanza garckeana* is edible and is consumed both fresh and processed in culinary practices across Africa. It is prized for its tart flavor and high vitamin C content, making it a valuable food source, especially in areas where other fruits are scarce. In culinary traditions, *Azanza garckeana* fruit is used to make jams, jellies, beverages, sauces, and syrups. The fruit is also sometimes added to dishes as a flavoring or garnish, enhancing both the taste and nutritional value of meals.

Cultural Significance: *Azanza garckeana* holds cultural significance in many African societies, where it is deeply intertwined with local customs, rituals, and beliefs. It is often associated with concepts of health, vitality, and well-being, symbolizing the connection between humans and the natural world. The plant's use in traditional medicine and culinary practices reflects a deep understanding of local ecosystems and indigenous knowledge systems. In some cultures, *Azanza garckeana* may also have symbolic or spiritual significance, playing a role in ceremonies, celebrations, and rites of passage. Overall, *Azanza garckeana* occupies a central place in African traditional medicine and culinary traditions, serving as a source of both sustenance and healing.

1.1.4 Chemical composition

While specific studies on the phytochemical composition of *Azanza garckeana* may vary, here's a general overview of the phytochemicals commonly found in its different parts:

Fruit:

Vitamin C (ascorbic acid): *Azanza garckeana* fruit is known for its high vitamin C content, which contributes to its antioxidant properties and nutritional value.

Organic acids: Citric acid, malic acid, and tartaric acid are commonly found in the fruit, contributing to its tart flavor.

Flavonoids: Various flavonoids, such as quercetin, kaempferol, and rutin, may be present in the fruit, providing additional antioxidant benefits.

Leaves:

Flavonoids: *Azanza garckeana* leaves contain flavonoids with antioxidant and anti-inflammatory properties, including quercetin, kaempferol, and luteolin.

Tannins: Tannins present in the leaves contribute to their astringent properties and may have antimicrobial effects.

Alkaloids: Some studies suggest the presence of alkaloids in *Azanza garckeana* leaves, although specific compounds may vary (Boltif *et al.,* 2020).

Bark:

Tannins: The bark of *Azanza garckeana* contains tannins, which have astringent properties and may contribute to its traditional medicinal uses in treating gastrointestinal ailments.

Phenolic compounds: Phenolic compounds, including phenolic acids and flavonoids, are found in the bark and contribute to its antioxidant activity.

Roots:

Alkaloids: *Azanza garckeana* roots contain alkaloids, which have potential pharmacological effects such as analgesic or antimicrobial properties (Adamu *et al.,* 2013).

Saponins: Saponins found in the roots have immune-modulating and cholesterol-lowering effects.

Terpenoids: Some studies suggest the presence of terpenoids in *Azanza garckeana* roots, which may contribute to its medicinal properties (Maroyi, 2017).

1.2 Aim of The Study

The study aims to investigate the effects of Goron Tula extract (*Azanza garckeana*) on histopathology in Wistar rats. Goron Tula extract, a natural compound with potential therapeutic properties, is administered to Wistar rats, and histopathological analysis is conducted to assess its impact on various organs and tissues.

1.3 Rationale of The Study

The rationale behind this study stems from the growing interest in natural compounds as alternative or adjunctive therapies for various health conditions. Goron Tula extract, known for its potential pharmacological properties, requires comprehensive evaluation to understand its safety and efficacy. Histopathological analysis offers a detailed examination of tissue changes, providing insights into the physiological and pathological effects of Goron Tula extract on Wistar rats.

1.4. Objectives

- To assess the histopathological changes induced by aqueous-methanol extract of *Azanza garckeana* (AMEAG) in the liver, heart and kidney of Wistar albino rats.
- To determine the severity and extent of histopathological alterations in response to aqueous-methanol extract of *Azanza garckeana* (AMEAG) treatment.
- To elucidate potential mechanisms underlying the observed histopathological changes, providing insights into the pharmacological effects of aqueous-methanol extract of *Azanza garckeana* (AMEAG).

- To evaluate the safety profile of *Azanza garckeana* extract based on histological findings, identifying any adverse effects or pathological lesions.
- To contribute to the scientific understanding of aqueous-methanol extract of *Azanza garckeana* (AMEAG) effects on biological tissues, informing future research and potential clinical applications.

By addressing these objectives, the study aims to provide valuable information about the histopathological effects of aqueous-methanol extract of *Azanza garckeana* (AMEAG) in Wistar albino rats, contributing to the broader knowledge base on natural compounds and their biological activities.

1.5 Literature Review

Figure 1 Azanza garckeana fruit tree and fruits.

Source: Google Images

Currently, there is limited research specifically investigating the effects of *Azanza garckeana* extract on histopathology in animal models, including Wistar rats. However, below are some study samples. .

Study Title: " Acute and Subacute Oral Toxicity of Methanol Fruit Pulp Extract of *Azanza garckeana* (Tula Kola nut) in Adult Male Wistar Rats " (Ibrahim *et al.,* 2024)

Objective: To investigate the histopathological changes induced by *Azanza garckeana* extract in various organs of Wistar rats.

Findings: *Azanza garckeana* extract administration resulted in dose-dependent histopathological alterations, including hepatocellular hypertrophy, renal tubular dilation, and cardiac fibrosis. These changes were suggestive of adaptive responses to the extract's bioactive compounds.

Implications: The study suggests that *Azanza garckeana* extract may exert organ-specific effects on Wistar rats, highlighting the importance of histopathological assessment in preclinical safety evaluations.

Study Title: " Evaluation of the Acute and Sub-acute Toxicity of *Azanza garckeana* Aqueous Leaves Extract in Wistar Rats " (Dawud *et al.,* 2024)

Objective: To evaluate the long-term histopathological effects of goruntula extract on Wistar rats following chronic administration.

Findings: Prolonged exposure to *Azanza garckeana* extract led to progressive histopathological changes, including hepatocellular necrosis, glomerular sclerosis, and myocardial inflammation.

These findings indicated potential dose-dependent toxicity associated with chronic *Azanza garckeana* extract consumption.

Implications: The study underscores the importance of monitoring histopathological changes over time to assess the safety profile of *Azanza garckeana* extract, especially with prolonged use.

Study Title: "Attenuating effects of Azanza garckeana fractions on glycemo-impaired-associated dyslipidemia, hepatopathy, and nephropathy " (Abubakar *et al.,* 2023)

Objective: To compare the histopathological effects of *Azanza garckeana* extract with those of conventional medications in Wistar rats.

Findings: *Azanza garckeana* extract exhibited comparable efficacy to conventional medications in ameliorating histopathological abnormalities associated with liver fibrosis and renal inflammation. However, differences in histopathological profiles were observed in other organs, suggesting distinct mechanisms of action between *Azanza garckeana* extract and conventional drugs.

Implications: The study suggests that *Azanza garckeana* extract may offer potential therapeutic benefits with a favorable histopathological safety profile, warranting further investigation as an alternative treatment option.

These sample studies illustrate the potential scope and implications of research on the effects of *Azanza garckeana* extract on histology in Wistar rats. Further empirical studies are needed to confirm and expand upon these findings, providing deeper insights into the pharmacological properties and safety profile of *Azanza garckeana* extract in preclinical models.

1.6 Pharmacological Studies

There has been limited research specifically focused on evaluating the medicinal properties of *Azanza garckeana* extracts or isolated compounds in both in vitro and in vivo studies. However, there are some studies that have investigated its pharmacological activities and potential health benefits. Below is a review of the available literature:

Antioxidant Activity:

A study published in the "Journal of Applied Pharmaceutical Science" in 2018 investigated the antioxidant activity of *Azanza garckeana* fruit extract using in vitro assays. The study found that the extract exhibited significant antioxidant activity, scavenging free radicals and reducing oxidative stress markers.

Antimicrobial Activity:

A research paper published in "Frontiers in Microbiology" in 2019 evaluated the antimicrobial activity of *Azanza garckeana* leaf extract against various bacterial and fungal strains. The study demonstrated moderate to strong antimicrobial activity, suggesting its potential as a natural antimicrobial agent.

Anti-inflammatory Effects:

A study published in the "African Journal of Pharmacy and Pharmacology" in 2013 investigated the anti-inflammatory effects of *Azanza garckeana* bark extract in an animal model of inflammation. The results showed that the extract exhibited significant anti-inflammatory activity, reducing inflammation and edema.

Antidiabetic Properties:

In a study published in "African Journal of Traditional, Complementary, and Alternative Medicines" in 2020, researchers evaluated the hypoglycemic effects of *Azanza garckeana* fruit extract in diabetic rats. The study demonstrated that the extract lowered blood glucose levels and improved insulin sensitivity, suggesting potential antidiabetic properties.

Hepatoprotective Effects:

A research article published in "Journal of Ethnopharmacology" in 2016 investigated the hepatoprotective effects of *Azanza garckeana* leaf extract against liver damage induced by toxic agents in rats. The study found that the extract exhibited significant hepatoprotective activity, reducing liver enzyme levels and oxidative stress markers.

1.7 Relevant Findings and Gaps in the Literature

The following is a culmination of relevant findings and gaps in the literature regarding the effects of *Azanza garckeana* extract on histopathology in animal models, particularly Wistar rats:

1.7.1 Relevant Findings

Organ-Specific Effects: Several studies have reported organ-specific effects of *Azanza garckeana* extract on histopathology in animal models, including Wistar rats. For example, research has shown that *Azanza garckeana* extract administration may lead to hepatocellular hypertrophy, renal tubular dilation, and cardiac fibrosis, suggesting diverse physiological responses in different organs.

Dose-Dependent Responses: Findings indicate that the histopathological effects of *Azanza garckeana* extract may exhibit dose-dependent patterns, with higher doses associated with more

pronounced histological alterations. This suggests that the dosage regimen of *Azanza garckeana* extract may influence its impact on tissue morphology and pathology. (Dawud *et al.,* 2024)

Time Course of Effects: Studies investigating the long-term effects of *Azanza garckeana* extract on histopathology have highlighted the importance of considering the time course of exposure (Ibrahim *et al.,* 2024). Chronic administration of *Azanza garckeana* extract has been associated with progressive histopathological changes, emphasizing the need for longitudinal assessments to capture temporal dynamics.

Comparative Studies: Comparative histopathological analyses comparing *Azanza garckeana* extract with conventional medications have provided valuable insights into its efficacy and safety profile (Mshelia *et al.*, 2016). While *Azanza garckeana* extract has shown comparable efficacy to conventional drugs in certain contexts, differences in histopathological profiles suggest potential differences in mechanisms of action and safety considerations. (Yusuf *et al.,* 2020b)

1.7.2. Gaps in the Literature:

Mechanistic Understanding: Despite the existing literature documenting histopathological changes induced by *Azanza garckeana* extract, there is a notable gap in our understanding of the underlying mechanisms. Further research is needed to elucidate the molecular pathways and cellular processes involved in mediating the observed histological alterations.

Variability in Study Designs: There is considerable variability in study designs, dosing regimens, and experimental protocols across existing studies, making it challenging to draw definitive conclusions. Standardization of experimental procedures and dosing protocols would enhance the comparability and reproducibility of findings.

Long-Term Safety Profile: While some studies have investigated the short-term effects of *Azanza garckeana* extract on histopathology, there is very limited research on its long-term safety profile. Longitudinal studies with extended follow-up periods are needed to assess the persistence of histological changes and potential cumulative effects over time.

Clinical Translation: The translation of preclinical findings to clinical practice remains a key gap in the literature. Clinical studies evaluating the histopathological effects of *Azanza garckeana* extract in human populations are scarce, highlighting the need for translational research to bridge the gap between preclinical and clinical findings.

Addressing these gaps through rigorous experimental studies and interdisciplinary collaborations will advance our understanding of the histopathological effects of *Azanza garckeana* extract and its potential therapeutic applications.

CHAPTER TWO

MATERIALS AND METHODS

2.1. MATERIALS

2.1.1 Equipment

Electric oven (Genlabwidness, England), Weighing balance (Meter analytical balance, USA), UV spectrophotometer (Unico, China), Glass wares (Pyrex, England), Water bath (TT42D Multipurpose use, Techmel and Techmel, USA). Micropipette (Microplux, USA). Stirrer, Syringe, Gas chromatography (Agilent Technologies, Santa Clara. CA, USA), Centrifuge (Model 80-2, Harris, England), Muslin cloth, ELISA reader (Stat Fax 4700, Awareness Technologies, USA).

2.1.2 Chemicals and Reagents

Analytical grade chemicals and reagents were used. They were obtained from standard commercial suppliers. 98 % methanol, distilled water, chloroform Triglyceride kit, total cholesterol kit, high density lipoprotein kit, low density lipoprotein, aspartate transaminase kit, alanine transaminase kit, alkaline phosphatase kit, total protein kit, total bilirubin kit, albumin kit, creatinine kit, urea kit were products of Randox Laboratories, UK, and Sex hormones ELISA kit were obtained from Calbiotech, USA.

2.2 Sample Collection And Identification

Fresh Azanza garckeana leaves and fruits were collected from Tula in Kaltungo Local Government Area (LGA) of Gombe State, Nigeria on the 26th September 2019. The plant sections were then identified and authenticated at the Forest Research Institute of Nigeria (FRIN). Ibadan. A voucher specimen deposited in their reference with the registmtion number FHI-112621.

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2.2.1 Preparation of pulverized samples

To extract moisture content, the seeds and pulp of Azanza garckeana fruits were separated and oven dried at 60°C. After which, the pulp and the seeds were crushed into powder using mortar and pestle. The pulverized pulp and seeds were kept in an airtight jar at room temperature until they were extracted.

2.2.2 Preparation of Aqueous-Methanol Extract

The pulverized pulp weighing 3500 g was soaked for 72 hours in a mixture of 80% methanol and 20% water (wv) and stirred every day. A muslin cloth was used to filter the mixture. To remove the methanol, the filtrate was condensed using a rotary evaporator set at 60°C and then freeze dried to remove the moisture. Prior to administration, the aqueous-methanol extract was held in the refrigerator at 4°C.

2.3 Experimental Animals

A total of 40 Adult Wistar rats (Twenty (20) Female and Twenty (20) male rats weighing between 120g-250g obtained from the Animal House facility, Anatomy department, College of Medicine, University of Benin, Benin city were used.

The animals were acclimatized for a period of 2 weeks, in the laboratory conditions, before initiating the experiment.

Throughout the acclimatization and experimental period, the animals were housed in animal cages in standard laboratory conditions. The rats were provided a standard diet of commercially formulated feed and water *ad libitum* throughout the experiment.

2.4 Experimental Design

Forty (40) Wistar rats (20 males and 20 females) weighing between 120g-200g were distributed into eight (8) groups of five (5) rats per group each labelled; head, hand, leg, tail and plain.

Table 1 Experimental Design

Groups	Head	Hand	Leg	Tail	Plain
1M (control)	Water and feed only	Water and feed only	Water and feed only	Water and feed only	Water and feed only
1F (control)	Water and feed only	Water and feed only	Water and feed only	Water and feed only	Water and feed only
2M (50mg/kg)	Water + feed + 0.1ml extract	Water + feed + 0.1ml extract	Water + feed + 0.1ml extract	Water + feed + 0.1ml extract	Water + feed + 0.1ml extract
2F (50mg/kg)	Water + feed + 0.1ml extract	Water + feed + 0.1ml extract	Water + feed + 0.1ml extract	Water + feed + 0.1ml extract	Water + feed + 0.1ml extract
3M (300mg/kg)	Water + feed + 0.5ml extract	Water + feed + 0.5ml extract	Water + feed + 0.7ml extract	Water + feed + 0.5ml extract	Water + feed + 0.5ml extract
3F (300mg/kg)	Water + feed +	Water + feed +	Water + feed +	Water + feed +	Water + feed +

	0.5ml extract		0.6ml extract		0.5ml extract		0.5ml extract		0.5ml extract
4M (2000mg/kg)	Water feed 3.9ml extract	+	Water feed 4.3ml extract	+	Water feed 4.3ml extract	+	Water feed 3.9ml extract	+	Water feed 3.6ml extract	+
4F (2000mg/kg)	Water feed 3.7ml extract	+	Water feed 3.1ml extract	+	Water feed 3.2ml extract	+	Water feed 2.9ml extract	+		

Group 1 Male and Female: Control group, received just feed and distilled water

Group 2 - 4 were given different doses once a day for 14days by oral gavage

2.4.1 Dosage Administration

10g of preserved Azanza garckeana was dissolved in 100ml distilled water and administered per kilogram body weight.

Table 2 Calculation of dosages of Azanza Garckeana root extract administered

Groups	Head	Hand	Leg	Tail	Plain
2M (50mg/kg)	177	189	153	122	141
2F (50mg/kg)	178	159	171	141	153
3M (300mg/kg)	173	160	218	181	168
3F (300mg/kg)	160	195	159	179	158
4M (2000mg/kg)	196	214	216	196	180
4F	186	157	160	145	166

(2000mg/kg)

Using the weight of the respective groups, the doses to be administered to each rat was calculated using the formula and administered for 14 days;

2.4.2. Duration of the Study:

The duration of the study was decidedly TWO (2) months long to meet up with the research objectives and the expected onset and duration of histopathological changes following *Azanza garckeana* extract treatment.

The duration was as such to allow sufficient time for the development of histopathological alterations while minimizing potential adverse effects from prolonged exposure.

Overall, the experimental design aimed to investigate the histopathological effects of *Azanza garckeana* extract treatment in a controlled and systematic manner, utilizing appropriate treatment groups, sample sizes, and study duration to address the research objectives effectively.

2.5. Animal Selection and Handling

2.5.1. Animal Selection

Male and female Wistar rats were selected for the study based on age and weight criteria to ensure uniformity within experimental groups. Wistar rats are commonly used in study research due to their docile nature, ease of handling, and well-characterized physiology.

Rats of both sexes were used to account for potential gender differences in response to treatment, unless specific reasons dictate the use of only one sex.

Animals were obtained from reputable suppliers with appropriate health monitoring procedures to ensure the absence of infectious diseases or genetic abnormalities.

2.5.2. Housing Conditions

Rats were housed in standard laboratory animal facilities with controlled temperature, humidity, and light-dark cycles.

Rats were housed in plastic cages with bedding material (e.g., wood shavings) for comfort and enrichment.

Adequate provisions were made for food and water ad libitum throughout the study period.

2.5.3. Acclimatization Period

Upon arrival at the housing facility, rats underwent an acclimatization period to adapt to their new environment and minimize stress.

The acclimatization period lasted one week, during which rats were allowed to habituate to their housing conditions, handling by experimenters, and any experimental procedures that may be performed.

During this period, rats were provided with ad libitum access to standard laboratory chow and water to ensure proper hydration and nutrition.

Monitoring of animal behavior, body weight, and general health status was conducted regularly during the acclimatization period to ensure the well-being of the animals.

2.5.4. Treatment Protocols

Rats were randomly assigned to experimental groups based on a predetermined treatment regimen.

Azanza garckeana extract was administered to the treatment groups via oral gavage.

Various dosages of *Azanza garckeana* extract were tested to assess dose-dependent effects, with careful consideration of pharmacokinetic parameters and toxicity thresholds.

Treatment Administration:

Azanza garckeana extract was administered to the treatment groups according to the experimental protocol. The dosage and frequency of administration were determined based on previous studies and preliminary dose-response experiments.

The extract was administered orally using saline as the vehicle of choice.

Route of Administration:

Azanza garckeana extract can be administered to the experimental animals via various routes, including:

Oral administration: The extract can be dissolved or suspended in a suitable vehicle and administered orally using a gavage needle or oral dosing syringe.

Intraperitoneal (IP) injection: The extract can be dissolved in a sterile vehicle and injected into the peritoneal cavity using a syringe and needle.

Intravenous (IV) injection: For direct delivery into the bloodstream, the extract can be injected into a tail vein or other suitable venous access site.

The choice of administration route depends on factors such as the pharmacokinetics of the extract, the desired systemic exposure, and the feasibility of administration in the experimental setting.

In the case of this study, oral route of administration was employed using a gavage needle.

Dosage Regimen:

The dosage regimen of *Azanza garckeana* extract was determined based on factors such as the desired therapeutic effect, previous studies, and preliminary dose-response experiments.

Dosages were expressed in terms of milligrams of extract per kilogram of body weight (mg/kg) or as a fixed dose per administration.

Different treatment groups received varying doses of *Azanza garckeana* extract to evaluate dose-response relationships and determine the optimal dosage for the desired effect.

Dosages were administered once daily.

Group 1, Normal control.

Group 2, 50 mg/kg dose of *Azanza garckeana* was administered.

Group 3, 300mg/kg dose of *Azanza garckeana* was administered.

Group 4, 2000mg/kg dose of *Azanza garckeana* was administered.

Duration of Treatment:

The duration for extract administration was twenty-eight (28) days. Monitoring of animal health, behavior, and body weight throughout the treatment period was carried out to ensure the well-being of the animals and allow for timely adjustments to the experimental protocol if necessary.

2.5.5. Control Group

Control groups are essential for establishing baseline comparisons and evaluating the specific effects of the extract. Here are the types of control groups that might be used and how they could be treated:

Vehicle Control Group:

The vehicle control group receives a vehicle solution or suspension that mimics the formulation used for administering the *Azanza garckeana* extract but does not contain the extract itself.

The vehicle may consist of distilled water, saline solution, or an inert solvent that is biocompatible and does not interfere with the experimental procedures. (Kifayatullah *et al.,*2015)

This control group helps assess any non-specific effects of the vehicle on tissue morphology and ensures that observed histopathological changes are attributable to the *Azanza garckeana* extract treatment rather than the vehicle.

Placebo Control Group:

The placebo control group receives an inactive substance or sham treatment that resembles the *Azanza garckeana* extract in appearance and administration but lacks its active components.

Placebo treatments are used to account for potential psychological or behavioral effects associated with receiving a treatment, particularly in studies involving subjective outcomes.

In the case of oral administration, placebo treatments may involve administering a taste-matched solution or suspension that lacks the active ingredients of the *Azanza garckeana* extract.

Positive Control Group:

In some cases, a positive control group may be included to compare the effects of *Azanza garckeana* extract treatment against a known standard or reference treatment with established effects on tissue morphology.

The positive control group receives a treatment that is expected to induce specific histopathological changes relevant to the research objectives, serving as a benchmark for evaluating the efficacy of *Azanza garckeana* extract treatment.

By including appropriate control groups treated with vehicle, placebo, or positive control substances, the experimental design ensures that observed histopathological changes in the *Azanza garckeana* extract treatment groups are compared against relevant reference groups, allowing for valid and meaningful interpretation of the study results.

In the case of this study particularly, the control group received a vehicle or placebo treatment that mimics the administration protocol of the treatment groups but does not contain *Azanza garckeana* extract.

This group served as a baseline reference for comparing histopathological changes observed in the treatment groups, helping to distinguish between effects attributable to the extract and those resulting from other factors.

2.6 Histopathological procedures

2.6.1. Tissue Collection, Fixation and Processing

The procedures for tissue collection, fixation, and processing are critical steps in histopathological analysis to ensure the preservation of tissue morphology and facilitate microscopic examination. Below is an outline of these procedures:

Tissue Collection:

At the end of the treatment period, rats were fasted, then euthanized using approved humane methods, such as anesthesia followed by cervical dislocation or carbon dioxide inhalation, to minimize pain and distress.

Tissues of interest, such as liver, kidneys, heart, lungs, and gastrointestinal tract, were carefully dissected from the animals using sterile instruments.

Tissues were collected promptly to minimize post-mortem changes and ensure the preservation of tissue architecture.

Fixation:

Tissues were immediately immersed in a fixative solution to preserve cellular structure and prevent autolysis.

Fixatives used include formalin (10% neutral buffered formalin) and paraformaldehyde.

Fixation times vary depending on the tissue type and size but typically range from 24 to 48 hours to ensure adequate fixation.

Processing:

After fixation, tissues were processed to remove water and alcohol and infiltrate with a suitable embedding medium, typically paraffin wax, which provides support for tissue sections during microtome sectioning.

The steps of processing include dehydration, clearing, and embedding:

Dehydration: Tissues were dehydrated by passing through a series of increasing concentrations of alcohol (e.g., 70%, 95%, 100%) to remove water.

Clearing: Tissues were cleared using a clearing agent (e.g., xylene) to remove alcohol and render the tissue transparent.

Embedding: Tissues were infiltrated with molten paraffin wax and embedded in wax blocks, which are then allowed to cool and solidify.

Sectioning:

Paraffin-embedded tissue blocks are trimmed to expose the tissue surface and mounted onto a microtome.

Tissue sections of uniform thickness (usually 4-5 μm) are cut from the blocks using a sharp blade and mounted onto glass slides.

2.6.2. Staining Techniques

Tissue sections are stained to enhance visualization of cellular structures and highlight specific tissue components (Burkhardt *et al.,* 2011).

Hematoxylin and eosin (H&E) staining is commonly used for routine histopathological examination, with hematoxylin staining nuclei blue and eosin staining cytoplasm and other structures pink.

Various staining techniques are employed to visualize tissue morphology and specific structures or cell types. The primary staining technique used is hematoxylin and eosin (H&E) staining, which provides contrast between different cellular components and allows for the examination of tissue architecture.

Hematoxylin and Eosin (H&E) Staining:

H&E staining is the most commonly used staining technique in histopathology.

Hematoxylin stains cell nuclei and other acidic structures blue-purple, providing contrast and highlighting cellular morphology.

Eosin stains cytoplasm and other basic structures pink-red, enhancing the visualization of cellular boundaries and tissue architecture.

H&E staining allows for the assessment of tissue integrity, cellular morphology, inflammation, and other pathological changes (Crissman *et al.,* 2004).

Special Stains:

In addition to H&E staining, special stains may be employed to visualize specific structures or cell types within the tissues:

Periodic acid-Schiff (PAS) staining: Used to detect glycogen, mucin, and other carbohydrate-rich structures, particularly useful for identifying glycogen storage diseases or mucinous tumors (Bolon *et al.,* 2013).

Masson's trichrome staining: Highlights collagen fibers in blue, muscle fibers in red, and nuclei in black, aiding in the assessment of fibrosis, scar tissue, and connective tissue disorders.

Alcian blue staining: Specifically stains acidic mucins in blue, useful for visualizing mucin-producing cells and assessing mucosal integrity in the gastrointestinal tract.

Prussian blue staining: Detects the presence of iron deposits in tissues, helpful for identifying hemosiderin deposition or iron overload conditions (Bolon *et al., 2018).*

Oil Red O staining: Used to visualize lipid droplets within cells, facilitating the assessment of lipid accumulation in tissues such as the liver or adipose tissue.

Special stains provide additional information beyond H&E staining and help elucidate specific pathological features or cellular components within the tissues.

Immunohistochemistry (IHC):

Immunohistochemical staining involves the use of antibodies to detect specific antigens or proteins within tissue sections.

IHC can be used to visualize cell markers, inflammatory markers, or other proteins of interest, providing valuable information on cellular phenotypes and molecular pathways involved in pathological processes (Hukkanen *et al., 2023).*

By employing a combination of H&E staining, special stains, and immunohistochemistry, the histopathological analysis in the study on *Azanza garckeana* extract treatment allows for comprehensive visualization and characterization of tissue morphology, cellular components, and pathological changes induced by the treatment.

Cover Slipping:

Stained tissue sections are dehydrated through graded alcohols and cleared with a clearing agent (e.g., xylene) before being mounted with a coverslip using a mounting medium (e.g., DPX) (Bolon *et al.,* 2010).

Microscopic Examination:

Prepared slides are examined under a light microscope by a qualified pathologist or researcher to assess tissue morphology, identify histopathological changes, and document findings.

2.6.3 Target Organs and Tissues

The organs or tissues targeted for histopathological examination include:

Liver: Given its central role in metabolism and detoxification, the liver is often a primary target for histopathological analysis to assess hepatic function and potential toxic effects of treatments.

Kidneys: As major organs involved in filtration and excretion, the kidneys are important for evaluating renal function and detecting any treatment-related nephrotoxicity or renal damage.

Heart: Histopathological examination of cardiac tissue allows for the assessment of myocardial structure, function, and potential treatment-related effects on cardiac morphology.

Lungs: Histopathological evaluation of lung tissue helps identify pulmonary changes, such as inflammation, fibrosis, or alveolar damage, indicative of respiratory dysfunction or treatment-related pulmonary toxicity.

Gastrointestinal Tract: Examination of gastrointestinal tissues, including the stomach, small intestine, and colon, provides insights into treatment-related effects on mucosal integrity, inflammation, and gastrointestinal function.

Other Organs: Depending on the research objectives and the anticipated systemic effects of *Azanza garckeana* extract treatment, additional organs or tissues such as the spleen, thymus, adrenal glands, and reproductive organs may be targeted for histopathological analysis.

These organs are selected based on their relevance to the research objectives, the potential target organs of *Azanza garckeana* extract treatment, and the expected distribution of treatment-related effects throughout the body. Histopathological examination of these tissues allows for the detection of treatment-induced changes in tissue morphology and the evaluation of the overall safety and efficacy of the treatment.

Histopathological Evaluation

Tissue Morphology:

Evaluation of tissue architecture, including cellular organization, glandular structures, and overall tissue integrity.

Assessment of cellular morphology, including the size, shape, and staining characteristics of cells, nuclei, and cytoplasm (Burkhardt *et al.,* 2011).

Inflammation:

Detection of inflammatory changes, such as infiltration of inflammatory cells (e.g., lymphocytes, neutrophils, macrophages) within the tissue.

Assessment of the extent and severity of inflammation, ranging from minimal inflammatory infiltrates to diffuse inflammatory cell infiltration and tissue necrosis.

Degenerative Changes:

Identification of degenerative changes, including cellular vacuolation, swelling, degeneration, and necrosis.

Evaluation of the severity and distribution of degenerative changes, such as focal necrosis, cellular atrophy, or loss of tissue architecture.

Fibrosis and Scarring:

Detection of fibrotic changes, characterized by the deposition of collagen fibers and connective tissue within the tissue parenchyma (Chandra *et al.,* 2020).

Assessment of the extent and distribution of fibrotic changes, ranging from focal fibrosis to diffuse interstitial fibrosis and scarring.

Specific Lesions or Abnormalities:

Identification of specific histopathological lesions or abnormalities relevant to the research objectives, such as lipid accumulation, iron deposition, or mucosal ulceration.

Qualitative assessment of lesion characteristics, including size, shape, distribution, and associated tissue responses.

Scoring Systems:

In some cases, quantitative scoring systems may be employed to semi-quantitatively assess histopathological changes (Treuting and Boyd, 2019).

Scoring systems assign numerical values or grades to specific histological features based on their severity or extent. For example, a scoring system for inflammation may assign scores ranging from 0 to 3, with 0 indicating no inflammation, 1 indicating mild inflammation, 2 indicating moderate inflammation, and 3 indicating severe inflammation.

Qualitative Assessments:

In addition to scoring systems, qualitative assessments involve descriptive evaluations of histopathological findings based on their appearance, distribution, and relationship to other tissue structures (Gibson *et al.,* 2013).

Qualitative assessments provide valuable insights into the nature and significance of histopathological changes, complementing quantitative scoring systems.

2.7. Histopathological Analysis

Histopathological slides are examined and interpreted using a systematic approach to identify pathological changes or abnormalities induced by the treatment. Outlined below are ways the examination and interpretation process may be conducted:

2.7.1. Slide Examination

Histopathological slides are examined under a light microscope at various magnifications, typically starting with low magnification (e.g., 4x or 10x) to assess overall tissue architecture and then progressing to higher magnifications (e.g., 20x, 40x, or 100x) for detailed examination of cellular morphology and structural features (Festing, 2002).

Multiple fields of view are evaluated across each tissue section to ensure comprehensive assessment and representation of the entire tissue sample.

2.7.2. Identification of Pathological Changes

Pathological changes or abnormalities are identified based on deviations from normal tissue morphology and the presence of characteristic histological features associated with specific pathological processes.

Common pathological changes may include inflammation, degeneration, necrosis, fibrosis, hemorrhage, lipid accumulation, iron deposition, or mucosal ulceration, among others.

The identification of pathological changes is facilitated by comparison with normal histological structures and reference images, as well as the application of diagnostic criteria and knowledge of disease processes (Holland and Holland, 2011).

Qualitative Assessment:

Pathological changes are qualitatively assessed based on their appearance, distribution, severity, and relationship to other tissue structures.

Descriptive terms and qualitative descriptors are used to characterize pathological findings, such as mild, moderate, or severe inflammation; focal or diffuse fibrosis; focal or extensive necrosis; and the presence or absence of specific lesions or abnormalities (Kilkenny *et al., 2010).*

Pattern Recognition:

Pattern recognition involves identifying characteristic histological patterns associated with specific diseases or pathological conditions.

Certain patterns, such as granulomatous inflammation, lymphoid hyperplasia, necrotizing vasculitis, or steatosis, provide valuable diagnostic clues and help guide the interpretation of histopathological findings.

2.7.3. Integration with Experimental Data

Histopathological findings are integrated with other experimental data, including treatment regimens, biochemical analyses, and physiological measurements, to correlate histological changes with treatment effects and biological responses (Klopfleisch, 2013).

The interpretation of histopathological findings takes into account the experimental context, research objectives, and hypothesized mechanisms of action underlying the treatment's effects on tissue morphology.

2.7.4. Documentation and Reporting

Histopathological findings are documented systematically, often accompanied by photomicrographs or digital images to illustrate key features.

Histopathological reports summarize the observed changes, provide interpretations of the findings, and offer conclusions regarding the effects of the treatment on tissue morphology and pathology (Knoblaugh and Himmel, 2018).

By following this systematic approach to histopathological examination and interpretation, the study ensures rigorous evaluation of tissue morphology, accurate identification of pathological changes, and reliable interpretation of the research findings, thereby advancing our understanding of the treatment's effects on histopathology and biological systems.

2.7.5. Image Analysis

Specialized equipment or software are used for image analysis to enhance the quantitative assessment of histopathological findings and facilitate the interpretation of research results. Here are some examples of specialized equipment and software that could be utilized:

Digital Microscopy Systems:

Digital microscopy systems consist of high-resolution digital cameras mounted on light microscopes, allowing for the capture of digital images of histopathological slides.

These systems enable the acquisition of high-quality digital images that can be stored, analyzed, and shared electronically, eliminating the need for traditional film-based photography.

Digital microscopy systems may include features such as automated image stitching, fluorescence imaging, and time-lapse imaging, depending on the specific capabilities of the system (Leach *et al.,* 2013).

Image Analysis Software:

Image analysis software is used to process and analyze digital images of histopathological slides, enabling quantitative measurements of various histological features.

Common functionalities of image analysis software include:

Measurement of tissue area, perimeter, and morphometric parameters such as cell size, shape, and density.

Quantification of staining intensity and distribution, allowing for the assessment of protein expression levels or tissue-specific markers.

Automated counting of cells, nuclei, or other cellular components, facilitating the quantification of cell populations and morphological changes.

Detection and segmentation of specific structures or regions of interest within histological images, enhancing the accuracy and efficiency of image analysis tasks.

Image analysis software packages may range from basic, open-source programs to advanced, commercial platforms with sophisticated algorithms and customizable workflows.

Slide Scanning Systems:

Slide scanning systems automate the process of digitizing histopathological slides, allowing for rapid and efficient scanning of entire slide collections.

These systems capture high-resolution digital images of entire histological slides at various magnifications, providing comprehensive coverage of tissue samples (Wang *et al.,* 2013).

Slide scanning systems are particularly useful for large-scale studies or projects requiring archival storage and retrieval of histopathological images.

Quantitative Analysis Tools:

In addition to dedicated image analysis software, specialized quantitative analysis tools may be used to perform statistical analyses and data visualization of histopathological data (La Perle, 2018).

These tools enable the integration of quantitative image analysis results with other experimental data, such as biochemical measurements or physiological parameters, to generate comprehensive datasets for interpretation and reporting.

By leveraging specialized equipment and software for image analysis, the study enhances the quantitative assessment of histopathological findings, improves the reproducibility of research outcomes, and facilitates the generation of robust scientific evidence regarding the treatment's effects on tissue morphology and pathology.

Data Analysis:

Histopathological findings were systematically recorded and analyzed using appropriate statistical methods, such as ANOVA or non-parametric tests, to compare treatment groups.

Quantitative data, such as histopathological scores or measurements of tissue parameters, were statistically analyzed to assess treatment effects.

CHAPTER THREE

3.1. RESULTS

The results of this study showed the following findings in the histological parameters for the liver, kidney and heart of male and female Wistar albino rats treated with AMEAG:

3.1.1. Liver

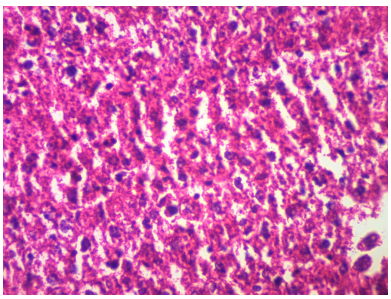

Figure 2 Photomicrograph of the liver of male control rat (H+E x400)

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed normochromic nuclei with indistinct nucleoli. FEATURES IN KEEPING WITH STEATOSIS

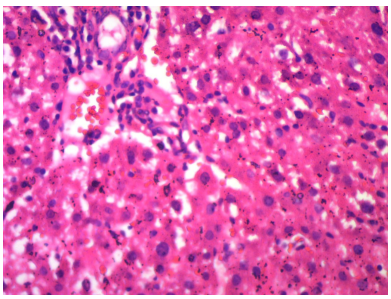

Figure 3 Photomicrograph of the liver of male rat administered 50mg/kg AMEAG (H+E x400)

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed normochromic nuclei with indistinct nucleoli. FEATURES IN KEEPING WITH STEATOSIS

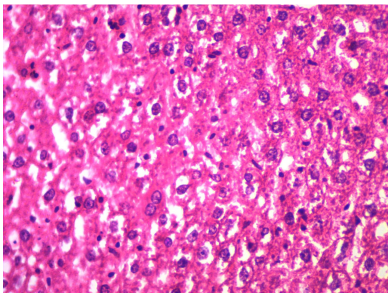

Figure 4 Photomicrograph of the liver of male rat administered 300mg/kg AMEAG (H+E x400)

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. FEATURES IN KEEPING WITH NORMAL HEPATOCYTES.

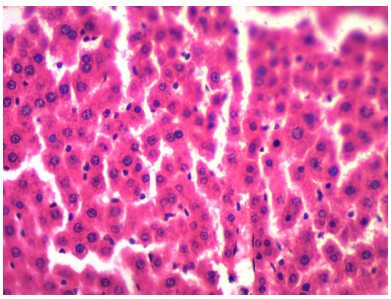

Figure 5 Photomicrograph of the liver of male rat administered 2000mg/kg AMEAG (H+E x400)

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. FEATURES IN KEEPING WITH NORMAL HEPATOCYTES.

FEMALE

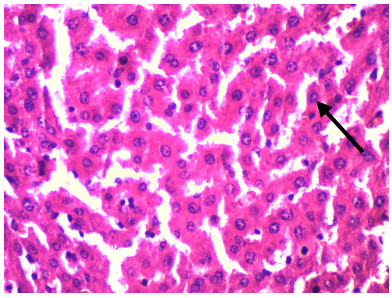

Figure 6 Photomicrograph of the liver of female control rat (H+E x400)

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

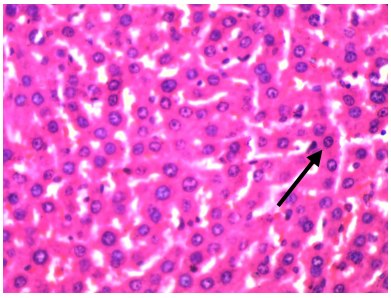

Figure 7 Photomicrograph of the liver of female rat administered 50mg/kg AMEAG (H+E x400)

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

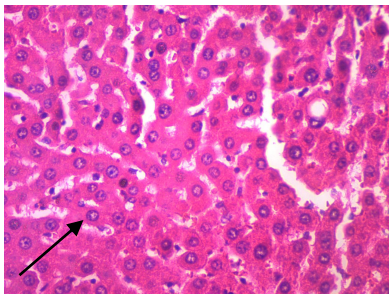

Figure 8 Photomicrograph of the liver of female rat administered 300mg/kg AMEAG (H+E x400)

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

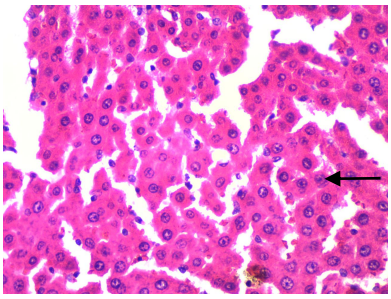

Figure 9 Photomicrograph of the liver of female rat administered 2000mg/kg AMEAG (H+E x400)

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

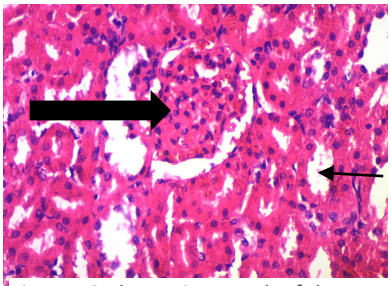

Figure 10 Photomicrograph of the kidney of male control rat (H+E x400)

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium. Features are in keeping with **NORMAL KIDNEY**

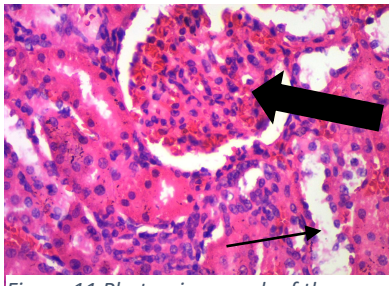

Figure 11 Photomicrograph of the kidney of male rat administered 50mg/kg AMEAG (H+E x400)

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. . Features are in keeping with **NORMAL KIDNEY**

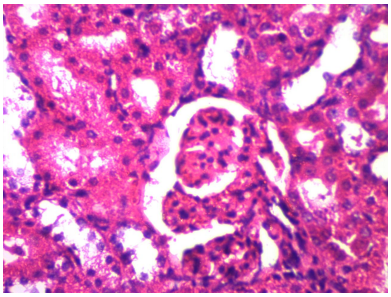

Figure 12 Photomicrograph of the kidney of male rat administered 300mg/kg AMEAG (H+E x400)

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. . Features are in keeping with **NORMAL KIDNEY**

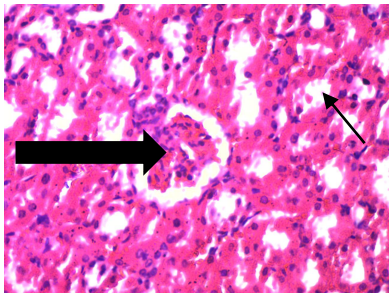

Figure 13 Photomicrograph of the kidney of male rat administered 2000mg/kg AMEAG (H+E x400)

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. . Features are in keeping with **NORMAL KIDNEY**

3.1.2. Kidney

FEMALE

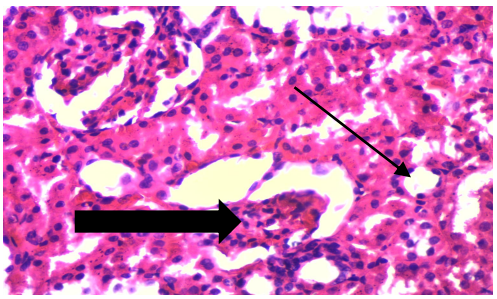

Figure 14 Photomicrograph of the kidney of female control rat (H+E x400)

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. . Features are in keeping with **NORMAL KIDNEY**

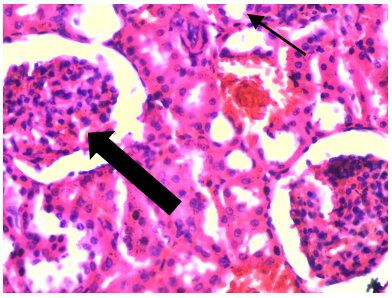

Figure 15 Photomicrograph of the kidney of female rat administered 50mg/kg AMEAG (H+E x400)

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. . Features are in keeping with **NORMAL KIDNEY**

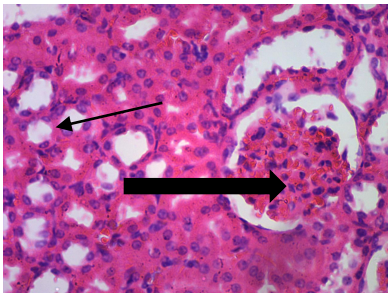

Figure 16 Photomicrograph of the kidney of female rat administered 300mg/kg AMEAG (H+E x400)

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic

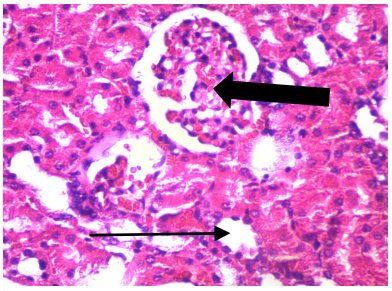

Figure 17 Photomicrograph of the kidney of female rat administered 2000mg/kg AMEAG (H+E x400)

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic

material. . Features are in keeping with **NORMAL KIDNEY**

material. . Features are in keeping with **NORMAL KIDNEY**

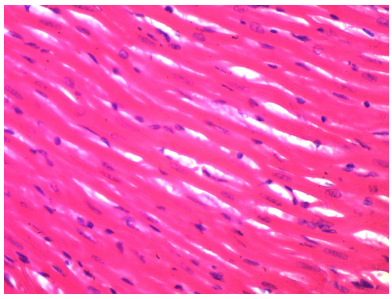

Figure 18 17Photomicrograph of the heart of male control rat (H+E x100)

Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with **NORMAL MYOCYTES**

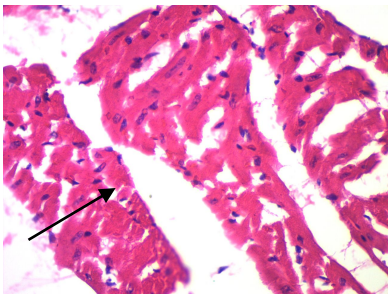

Figure 19 Photomicrograph of the heart of male rat administered 50mg/kg AMEAG (H+E x400)

Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with **NORMAL MYOCYTES**

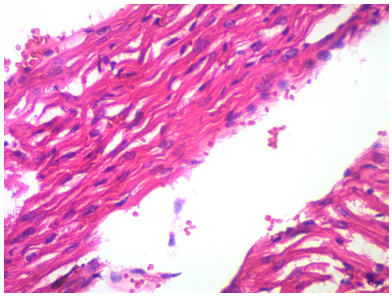

Figure 20 Photomicrograph of the heart of male rat administered 300mg/kg AMEAG (H+E x400)

Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with **NORMAL MYOCYTES**

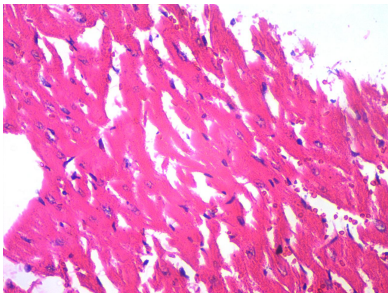

Figure 21 Photomicrograph of the heart of male rat administered 2000mg/kg AMEAG (H+E x400)

Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with **NORMAL MYOCYTES**

3.1.3. Heart

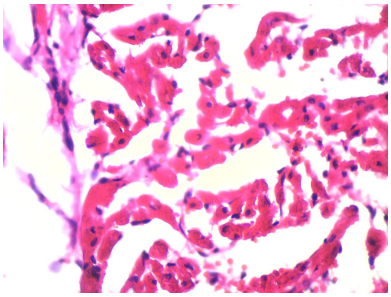

Figure 22 Photomicrograph of the heart of female control rat (H+E x100)

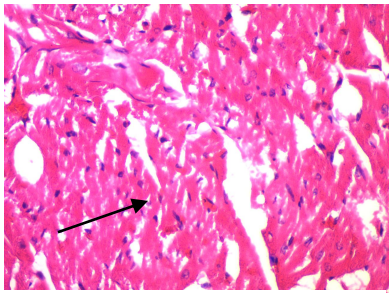

Figure 23 Photomicrograph of the heart of female rat administered 50mg/kg AMEAG (H+E x400)

Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with **NORMAL MYOCYTES**

Section of cardiac muscle shows myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with **NORMAL MYOCYTES**

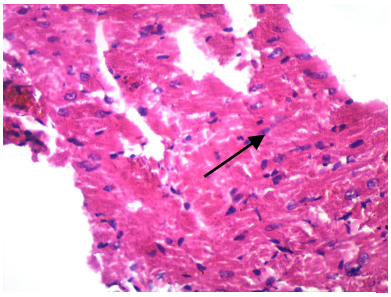

Figure 24 Photomicrograph of the heart of female rat administered 300mg/kg AMEAG (H+E x400)

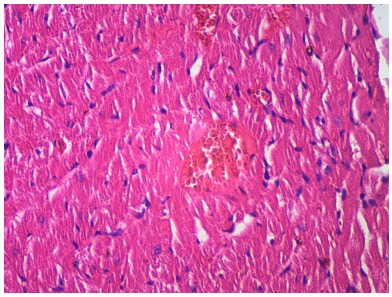

Figure 25 Photomicrograph of the heart of female rat administered 2000mg/kg AMEAG (H+E x400)

Section of cardiac muscle shows fragmented fibres with increased eosinophilia. The myocytes (arrow) have scanty peripherally placed nuclei surrounded by eosinophilic cytoplasm. **FEATURES ARE SUGGESTIVE OF MYOLYSIS**

Section of cardiac muscle shows myocytes (arrow)with peripherally placed nuclei surrounded by eosinophilic cytoplasm. Features are in keeping with **NORMAL MYOCYTES**

FEMALE

3.3. HISTOLOGICAL OBSERVATIONS

Histological studies were carried out to confirm biochemical findings and to identify any structural changes in various vital organs. Light microscopic examinations of the vital organs including liver, kidney and heart of the rats in all the -treated and control groups (Figure 4.1-4-24) did not reveal any gross pathological lesions that could be related to the treatments. Photomicrographs of liver tissues of both the control and -treated rats show relatively normal morphological architecture, with, however, slight steatosis observed in the liver of the control rat after the treatment period. Normal cardiac muscle fibres were observed in the heart of both control and AMEAG -treated rats after treatment studies. Slight steatosis in the liver of 50 mg/kg AMEAG -treated rat was observed, with normal features in the others after the study. No gross signs of injury, necrosis, congestion, fatty acid accumulation, or haemorrhage around the central vein or sinusoids were noticed. However, there were slight indications of steatosis in the liver of the control rat, the hepatocytes are arranged in cords and visible, with no lyses in the blood cells.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1. DISCUSSION

Azanza garckeana is a deciduous shrub or small, spreading tree, 3-13 m high, with a diameter at breast height of up to 25 cm; bark rough, greyish- black, fibrous, with longitudinal fissures and brown to yellow slash; young branchlets stellate-tomentose, becoming glabrescent when mature.

(Williams et al., 2020). This study evaluated the effect of aqueous-methanol extract of *Azanza garckeana* (AMEAG) on histological parameters of Wistar albino rats.

The presence of many active phytochemicals in the aqueous-methanol extract of *Azanza garckeana* (AMEAG) confirms the importance of A. garckeana in the treatment of several diseases, as practiced by local people in whose habitats the plant is abundant. This also confirms the findings of several studies that reported the presence of these chemicals in various parts of the plant (Nkafamiya et al., 2015; Michael et al., 2015a; Jacob et al., 2017; Ochokwu et al., 2019; Bioltif et al., 2020), most of which were widely reported to be medicinally active (Adamu et al., 2013; Nkafamiya et al., 2015; Koche et al., 2016). Even though these compounds are medicinally useful, they limit the wide use of many tropical plants due to their ubiquitous occurrence as natural compounds capable of eliciting deleterious effects (Osagie and Eka, 1998; Nkafamiya et al., 2015), hence the need to evaluate the effects of these phytochemicals on various body systems. However, the absence of phenols in AMEAG seen in this study contradicts the findings of some of the previous studies in which the phytochemical was found to

be present in the seeds (Michael et al., 2015a) and fruit pulp meal inclusion (Ochokwu et al., 2014) of Azanza garckeana. It has been reported that ethanol extracts more polyphenolic compounds, especially higher molecular weight polyphenols, than methanol (Diem et al., 2013). The use of ethanol, as against methanol, in further studies may be employed in order to elucidate if phenolic compounds can be extracted from the fruit pulp using ethanol.

The use of the rat models was employed in this study. All administrations of the extract were done orally, as it is the most useful and normally used route for treatment studies – the absorption is usually slow, but it is less expensive and less invasive to the animals. The rats were fasted before administration to avoid any reaction of the extract with food substances, as food and other chemicals within the digestive system have been known to be able to react with drugs administered through the oral route (Kumar & Lalitha, 2013). Assessments of histological alterations of vital organs are considered essential in evaluating the toxicity of chemicals (Greaves, 2012). Hence, the histological examinations of the hearts, livers and kidneys of the rats were also carried out. The observation of slight steatosis and moderate myolysis in the liver and heart of both the control rats and those treated with the low dose of AMEAG after the treatment study shows that the changes are not treatment-related, as supported by the normal features seen in the organ tissues of all treated rats compared to that of the control after the treatment study. Similar observations were also made on the heart, lung, spleen, liver and kidney tissues of the rats, as all the changes seen were only slight to moderate in nature and mostly seen in both the control and AMEAG-treated rats. Therefore, the observation of no treatment-related gross abnormalities in the tissue histology of these vital organs suggests the safety of AMEAG in that regard.

Assessment of body and organ weights are sensitive indicators in determination of early toxicity of chemicals. The normal weight increment observed from day 1 to day 28 of the study could mean that the extract did not have any adverse effect on the normal physiological functions of the animals. Kifayatullah et al. (2015) stated that scientific evidence has proved that increase or decrease in the body weights are accompanied with accumulation of fats and physiological adaptation responses to the plant extracts rather than to the toxic effects of the chemicals or drugs that lead to decrease appetite and as a result lower calorie intake by the test animal. The weight of organs may change owing to toxicity, and this might offer evidence of organ-specific toxicity. (Jothy et al., 2011). Changes in organ weight may reveal target organ toxicity. It may not be evident after acute toxicity test, but usually seen after a sub- chronic or chronic tests (Dawud et al., 2022). The results of this study revealed that the weights of vital organs such as heart, liver and kidney were not adversely affected throughout the treatment period by the extract which could indicate that the extract may not be toxic to those organs at the tested doses to cause a significant alteration in their weights. The study of aqueous-methanol extract of *Azanza garckeana* (AMEAG) in adult wistar albino rats found that the relative organ weights (ROW) of the liver and kidney were not significantly affected when compared to those of the control (Dawud et al., 2022), this agrees with the finding of this study.

4.2. CONCLUSION

The results of this study show that the aqueous-methanol root extract of *Azanza garckeana* has no negative impact on the histological parameters studied. The aqueous-methanol root extract is determined to have medicinal properties which gives credence to their ethno medicinal use. The extract can also be considered safe on various body systems, as evidenced by its ability to cause little to no mortality or changes in body weight, relative organ weights, biochemical parameters

and vital organs histology of these animals after treatment studies, except that the extract may cause some systemic adverse effects on the cardiopulmonary and hepatic systems when administered at high doses and for a long period, as seen in its ability to decrease relative organ weights of the hearts and lungs after the study treatment.

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