INVESTIGATING THE EFFECTS OF AQUEOUS FRUITS EXTRACT OF *Azanza garckeana* ON LEAD ACETATE-INDUCED TESTICULAR TOXICITY IN ADULT WISTAR RATS.

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CERTIFICATION

We certify that this work was carried out by **OROMAIWELE MAUREEN OSEMUDIAMEN** (**Matriculation number: PG/BMS1601677**) in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria in partial fulfilment of the award of M.Sc. in Anatomy under the supervision of **Dr. D. E. ODIASE**. All literature and other sources of information consulted, quoted or used in this research have been acknowledged and properly referenced.

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DEDICATION

This work is dedicated to God Almighty for keeping me alive throughout my academic journey and especially throughout the duration of this research.

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ABSTRACT

Azanza garckeana , a plant native to Central, Eastern, and Southern Africa, as well as parts of West Africa, is traditionally renowned for its medicinal properties, particularly its role in enhancing male reproductive health. This study investigated the protective effects of aqueous leaf extract of *Azanza garckeana* (AGE) against lead acetate-induced testicular toxicity in Wistar rats. This specific objectives evaluated the phytochemical constituents and antioxidant capacity of *Azanza garckeana* , as well as its effects on various physiological and biochemical parameters in Wistar rats. The objectives also included assessing changes in body and organ weights, oxidative stress markers, and male reproductive hormones (FSH, LH, testosterone) across experimental groups. Additionally, sperm analysis was conducted, and the impact of *A. garckeana* aqueous extract on the histology of testes in lead acetate-induced rats was examined. Thirty-six adult Wistar rats were divided into six groups. Group A served as the control, receiving only feed and water. Group B was exposed to 100 mg/kg body weight (BW) of lead acetate. Group C received 400 mg/kg BW of AGE only, while Group D was administered 800 mg/kg BW of AGE. Group E received a combination of 400 mg/kg BW of AGE and 100 mg/kg BW of lead acetate, and Group F received 800 mg/kg BW of AGE alongside 100 mg/kg BW of lead acetate. The maceration method was employed to extract the plant's bioactive components, as it is a simple and effective technique that ensures optimal recovery of phytochemicals while preserving their integrity for analysis. At the conclusion of the study period, the rats were weighed, sacrificed via scrotal incision, and their testes were harvested for analysis. Testicular weights were recorded, and biochemical assessments including oxidative stress markers, hormonal profiling, and sperm analysis were conducted. Histological evaluation of fixed testes was performed using hematoxylin and eosin staining. Statistical analyses were carried out using GraphPad Prism software (version 9), with data expressed as mean ± standard error of the mean (SEM), a method well-suited for handling small sample sizes and assessing variability within experimental groups. One-way analysis of variance (ANOVA), followed by Fisher's multiple comparisons post-hoc test, was employed to determine statistical significance ($P < 0.05$). Lead acetate exposure led to significant weight loss and oxidative stress, while damaging testicular cells and spermatogenesis. However, treatment with *Azanza garckeana* effectively mitigated these

effects, preserving testicular weight, antioxidant defenses, and cellular integrity, thereby supporting reproductive health.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF STUDY

Fertility is the ability of an individual or couple to conceive and establish a clinical pregnancy following at least 12 months of regular, unprotected sexual intercourse (Vander and Wyns, 2018). It is a critical aspect of reproductive health, influenced by a combination of genetic, physiological, environmental, and lifestyle factors. Infertility can be defined as the inability to achieve pregnancy within this timeframe, affects a significant portion of the global population, with an estimated 8% to 12% of reproductive-aged couples experiencing difficulties in conceiving. (Cox *et al*., 2022). Male infertility represents a complex medical condition influenced by multiple interacting factors, with male factors contributing to approximately 50% of all infertility cases when considering combined male-female reproductive interactions (Vander and Wyns, 2018). The underlying causes of male infertility are multifaceted, ranging from genetic abnormalities and nutritional deficiencies to environmental exposures, all of which can significantly impact testicular function and sperm quality (Sharma, 2017).

Genetic factors play a fundamental role in male fertility, with various inherited conditions directly affecting spermatogenesis and sperm function. Clinical manifestations often include oligospermia (reduced sperm count), asthenozoospermia (impaired sperm motility), and teratozoospermia (abnormal sperm morphology) (Oehninger and Kruger, 2021). Specific genetic disorders such as Y-chromosome microdeletions and Klinefelter syndrome (characterized by a 47,XXY chromosomal pattern) are particularly detrimental to testicular function, often leading to severe impairment of sperm production (Krausz and Rosta, 2020). These genetic conditions underscore the importance of comprehensive genetic evaluation in cases of unexplained male infertility (Akhavizadegan *et al.,* 2024).

While genetic predisposition establishes the baseline for reproductive potential, lifestyle and nutritional factors can significantly modify these genetic risks. A diet rich in specific nutrients has been shown to support male reproductive health, particularly for individuals with genetic vulnerabilities. Antioxidants such as vitamins C and E, selenium, and coenzyme Q10 play crucial roles in protecting sperm DNA from oxidative damage, which is especially important for men with genetic susceptibilities to sperm DNA fragmentation (Torres-Arce *et al*., 2021). Similarly, adequate intake of zinc and vitamin D is essential for maintaining proper testosterone levels, particularly in individuals with genetic tendencies toward hormonal imbalances (Awuchi *et al*., 2020). Folate, a B-vitamin critical for DNA synthesis and repair, assumes particular importance for men with mutations in genes involved in folate metabolism, such as the MTHFR gene, as deficiencies can exacerbate existing genetic risks for poor sperm quality (Liu *et al*., 2015).

Conversely, poor dietary choices and lifestyle factors can amplify genetic and environmental risks to male infertility. Excessive consumption of processed foods, diets high in saturated fats, and alcohol intake have been associated with increased oxidative stress and inflammation, which can further impair spermatogenesis in genetically susceptible individuals (Awuchi *et al*., 2020). Obesity, often linked to poor dietary habits, represents an additional risk factor that can disrupt hormonal balance, increase estrogen production, and negatively impact testicular function, thereby compounding existing genetic and nutritional challenges to fertility (Mazza *et al*., 2024).

Environmental toxicants present another significant threat to male reproductive health, with endocrine-disrupting chemicals (EDCs) being of particular concern. These environmental contaminants, which include pesticides, plasticizers, and industrial pollutants, can interfere with normal hormonal signaling and disrupt reproductive function (Piazza and Urbanetz, 2019). Among environmental pollutants, heavy metals such as lead have been extensively

studied for their detrimental effects on male reproduction. Lead exposure has been associated with testicular toxicity, reduced sperm quality, and hormonal imbalances, with lead acetate being a commonly studied model for understanding these effects (Gandhi *et al*., 2017). The mechanisms underlying lead-induced testicular damage include oxidative stress, inflammation, and direct histopathological changes to testicular tissue, effects that can lead to long-term reproductive dysfunction (Besong *et al*., 2024; Duruibe and Ogwuegbu, 2007). These environmental exposures can interact with genetic predispositions and nutritional status to create a complex web of factors influencing male fertility (Patisaul, 2009).

In light of growing concerns regarding heavy metal toxicity, there has been a surge in interest in natural products, particularly plant extracts, as potential therapeutic agents to alleviate the harmful effects of these environmental toxins. *Azanza garckeana* , also known as "*Goron Tula*" (Tula Kola nut) in Gombe and Morojwa in Botswana , is a plant native to Africa that has been traditionally utilized for its medicinal properties (Ochokwu *et al*., 2015). Preliminary studies indicate that *Azanza garckeana* extracts exhibit antioxidant, anti-inflammatory, and antibacterial activities, suggesting potential benefits in protecting against oxidative stress and organ toxicity (Nurudeen *et al.,* 2024). It is essential to explore the therapeutic potential of *Azanza garckeana* in mitigating heavy metal-induced toxicity. Specifically, the fruit extract of *Azanza garckeana* may offer protective effects against lead acetate-induced testicular damage.

Azanza garckeana , a plant species in the family Malvaceae, is widely distributed across tropical Africa (Sulieman, 2019). This plant has been traditionally utilized in the treatment of various ailments, including diabetes, hypertension, gastrointestinal disturbances, and infections (Lashani *et al.*, 2024). *Azanza garckeana* is also known for its potential to manage conditions such as malaria, respiratory disorders, wounds, and skin diseases (Dawud *et al*.,

2023). Additionally, the plant is recognized for its anthelmintic, diuretic, and anti-inflammatory properties, with applications in managing conditions like arthritis, dysentery, and menstrual irregularities (Deepti Katiyar *et al*., 2024)

The therapeutic significance of *Azanza garckeana* is attributed to its diverse phytochemical composition, which includes flavonoids, alkaloids, and tannins. These bioactive compounds contribute to its pharmacological activities, further supporting the plant's potential in addressing a range of health conditions. (Refaz Ahmad Dar *et al*., 2023).

1.2 STATEMENT OF RESEARCH PROBLEM.

Lead acetate exposure has been extensively studied for its detrimental effects on the male reproductive system. Research indicates that lead acetate can induce significant reproductive toxicity, leading to impaired spermatogenesis, hormonal imbalances, and structural damage to testicular tissue. In animal studies, lead acetate exposure has been shown to cause a significant decrease in epididymal sperm reserve, testicular sperm count, sperm motility, and sperm viability. These adverse effects are often accompanied by increased oxidative stress markers and decreased antioxidant enzyme activities in the testes.

Histopathological examinations reveal pronounced alterations in germ cells, including depletion of spermatogonia, spermatocytes, and spermatids, as well as damage to Leydig cells, which are crucial for testosterone production. Furthermore, lead exposure has been associated with reduced concentrations of androgens, such as testosterone, which play a vital role in maintaining male reproductive health. Elevated blood lead levels have been linked to impairments in spermatogenesis and hormonal disruptions, contributing to decreased fertility. These findings underscore the significant reproductive toxicity of lead acetate, highlighting the need for preventive measures and potential therapeutic interventions to mitigate its harmful effects on male reproductive health.

1.3 SIGNIFICANCE OF THE STUDY

The significance of this study lies in the potential benefits of using *Azanza garckeana* extract to mitigate the adverse effects of lead acetate on the male reproductive system in Wistar rats. The findings could contribute to the discovery of a natural remedy for alleviating lead acetate-induced reproductive toxicity and may pave the way for novel approaches to preventing and treating male infertility associated with lead exposure.

By exploring the protective properties of *Azanza garckeana* , this research aims to develop alternative therapeutic strategies that could reduce dependence on conventional treatments, which may have undesirable side effects. Additionally, these findings will enhance our understanding of lead acetate-induced reproductive toxicity and the potential mechanisms by which *Azanza garckeana* may counteracts its damaging effects.

Ultimately, this study will have a significant impact on the fields of toxicology, pharmacology, and male reproductive health, contributing to new strategies for safeguarding public health against environmental and industrial pollutants.

1.4 RESEARCH QUESTIONS

1. What are the phytochemical constituents and antioxidant capacity of *Azanza garckeana* that can potentially mitigate the toxic effect of lead acetate?
2. What are the proximate compositions of *Azanza garckeana* and how does the proximate compositions of *Azanza garckeana* relate to its potential to mitigate the toxic effects of lead acetate?
3. Can *Azanza garckeana* extract reduce the toxic effects of lead acetate on body and organ weight?
4. What are the possible mechanisms through which *Azanza garckeana* exerts its effects?

5. Can the aqueous extract of *Azanza garckeana* mitigate biochemical changes caused by lead exposure in the testes of adult Wistar rats?
6. Can *Azanza garckeana* extract protect the testes against lead acetate-induced oxidative stress?
7. Can the aqueous fruits extract of *Azanza garckeana* mitigate histological changes in the testes occasioned by lead exposure?

1.5 AIM AND OBJECTIVES OF THE STUDY

1.5.1 Aim of the study

The aim of this study is to investigate the potential therapeutic effects of aqueous fruits extract of *Azanza garckeana* on Lead acetate-induced testicular toxicity in Wistar rats.

1.5.2 Objectives of the study.

The above aim of study was achieved through two steps;

Step 1:

- The phytochemical constituents screening of *Azanza garckeana* .
- The quantitative analysis to determine the concentrations of identified phytochemicals in *Azanza garckeana* .
- The evaluation antioxidant capacity of *Azanza garckeana* using standardized assays.

Step 2:

- The objectives was to investigate the therapeutic effects of aqueous fruits extract of *Azanza garckeana* on Lead acetate-induced toxicity in;
- Changes in body and organ weight of experimental animals across all groups.
- Changes in hormone activities (Follicle stimulating hormones, luteinizing hormones and testosterone) of experimental animals across all groups.
- Changes in oxidative stress levels of experimental animals across all groups.
- Changes in sperm count, motility and morphology of experimental animals across all groups.
- Histological changes in testes of experimental animals across all groups.

CHAPTER TWO

LITERATURE REVIEW

2.1 TOXICANT: (LEAD ACETATE)

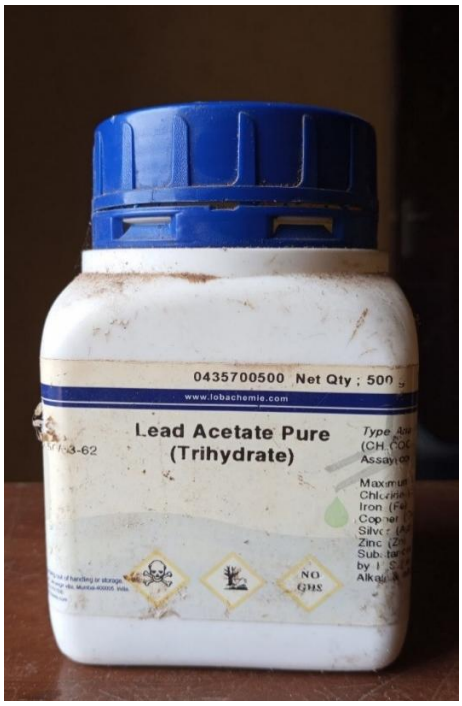


Photo taken by Maureen, 2024

Lead acetate is an inorganic molecule that can be found in the form of white, crystalline powder or granules. Historically known as sugar of lead, it has been widely used as a pigment, a mordant in dyeing and printing textiles, and as a catalyst in the production of polyvinyl chloride (PVC) (Yusuf *et al*., 2017). Nowadays, its use is regulated due to its toxicity and the availability of newer and more effective alternatives. Lead acetate has a relative molecular mass of 379.33 Dalton, a melting point of 280 C, and a boiling point of 200 C (Lide, 2005). It has a vapor pressure of 0.1 kPa at 136.2 C and a water solubility of 44.3 g/L, which increases to 460 g/L in boiling water; its solubility in alcohol is of 380 g/L (WHO, 2003). Lead acetate is currently used as a catalyst or reagent in several chemical reactions, and to a lesser extent as a pigment or stabilizer in the manufacture of lead-based products (Casas & Sordo, 2011) Potential sources of lead acetate intoxications are represented by lead-based paints,

contaminated soil and water, and certain types of traditional medicines, such as Ayurvedic and Unani remedies (Kamath *et al*., 2012; Kumar *et al*., 2015). Lead toxicity, as a result, is a significant clinical entity, as it is ubiquitous in the environment and poses serious risk to human health. The pathology of lead toxicity in humans includes direct damage to tissues and enzyme function as well as indirect damage as a result of oxidative stress. Despite this chemical compound being regulated, reports of intoxications and deaths, due to its use, have been reported in recent years.

2.1.1 Toxicokinetics

2.1.1.1 Absorption

Ingestion appears to be the most common route of exposure to lead acetate, but it can also be absorbed following dermal or inhalation exposure. The absorption of lead acetate has been demonstrated with numerous animal models. (Assi *et al*., 2016) estimated a lead acetate absorption of 5-10% in rats following a single oral dose of 10-50 mg/kg body weight. Similarly, an estimated absorption of 20-30% was calculated from single oral doses of 10-100 mg lead acetate/kg body weight in mice (Timchalk *et al*., 2006).

The rate of oral absorption of lead acetate in laboratory rodents has been shown to be related to age, intestinal pH, and diet (Endo *et al*., 1990). Indeed, young mice absorbed 40% of the orally administered lead acetate, whereas adult mice absorbed only 5% of the dose on standard diets. It has also been hypothesized that the nutritional status may contribute to the intestinal absorption of lead acetate, through competition with nutritionally essential divalent cations (e.g., Ca2+ and Zn2+) that may be characterized by insufficient body storage (WHO, 2003).

2.1.1.2 Distribution

After its absorption, lead acetate distributes widely to all tissues, predominantly the liver, the kidneys, the bones, and the central nervous system (CNS). This data mainly derives from

animal studies. Many authors found the liver and the kidneys to have the highest lead levels following repeated oral exposure to lead acetate in rats and mice (Kostial *et al*., 1978; Cikrt *et al.*, 1985). The brain has substantially lower lead levels after exposure to lead acetate; however, retention is the longest in this tissue.

Lead acetate has been shown to cross the blood-brain barrier, and its accumulation in the brain can lead to neurotoxic effects (Lidsky and Schneider, 2003). Furthermore, lead can also accumulate in human hair and nails following oral exposure to lead acetate (Foo *et al*., 1993).

Lead acetate has a limited ability to cross the placental barrier. This was shown by an intravenous study in pregnant rats (Patrick, 2006), in which, following intravenous doses of lead acetate (10-20 mg/kg) on a random day between days 9 and 17 of pregnancy, lead acetate was transferred inefficiently to the fetus, being blocked almost completely by the fetal membrane. The lead accumulated in the placenta and the yolk sac, but not in the amnion or the fetal body.

The distribution of lead acetate in the body is influenced by various factors, including age, sex, and nutritional status. For example, lead acetate has been shown to accumulate more readily in the bones of young animals than in adult animals (Kostial *et al*., 1978). Additionally, lead acetate has been shown to have a higher affinity for binding to proteins in the liver and kidneys than in other tissues (Cikrt *et al.,* 1985).

2.1.1.3 Metabolism

The available evidence indicates that the metabolism of lead acetate is similar for both humans and animals. Once absorbed, lead acetate is converted into lead ions, which can bind to various proteins and enzymes in the body. Lead ions can also be stored in the bones and released back into the bloodstream over time (ATSDR, 2007). The metabolism of lead acetate involves several enzymes, including delta-aminolevulinic acid dehydratase (ALAD), which is involved in the biosynthesis of heme (Finelli *et al*., 1975). Lead acetate has been

shown to inhibit the activity of ALAD, leading to an accumulation of delta-aminolevulinic acid (ALA) in the blood and urine (ATSDR, 2007). The excretion of lead acetate occurs mainly through the kidneys, with smaller amounts excreted through the feces and sweat (Klaassen, 2001). The half-life of lead acetate in the body is approximately 20-30 days, although this can vary depending on several factors, including the dose and duration of exposure (ATSDR, 2007).

2.1.2 Mechanisms of Toxicity

From a pathophysiological standpoint, the pervasive disruption of normal cell physiology by lead acetate is believed to derive from its avid covalent binding to sulfur, with lead ions replacing the hydrogen ion in the body's ubiquitous sulfhydryl groups. Lead acetate also reacts with phosphoryl, carboxyl, and amide groups, resulting in a widespread dysfunction of enzymes, membranes, transport mechanisms, and structural proteins. Lead acetate has been shown to cause oxidative stress, disrupt microtubule formation, impair protein and DNA synthesis, and disrupt synaptic transmission (ATSDR, 2007). Additionally, lead acetate has been shown to impair the immune response, disrupt calcium homeostasis, and compromise cell membrane integrity (Lidsky and Schneider, 2003). These alterations can lead to a range of toxic effects, including metabolic acidosis, vasodilatation, and shock.

The clinical manifestations of lead acetate toxicity involve multiple organ systems, including the nervous, hematopoietic, gastrointestinal, and renal systems (Heard, 2007). In particular, the toxicity of lead acetate to the developing brain is thought to result from its ability to disrupt synaptic transmission and impair neuronal development (Lidsky and Schneider, 2003). Additionally, lead acetate has been shown to cause anemia, nephropathy, and gastrointestinal disturbances, including abdominal pain, constipation, and diarrhea. The progression of testicular toxicity induced by lead acetate has been extensively studied in animal models. This process involves initial degenerative changes in the seminiferous tubules, such as

nuclear swelling, increased eosinophilia/basophilia, vacuolization, and cellular hypertrophy. In the early stages, these degenerative changes are accompanied by tubular regeneration. Occasionally, when there is minor toxic damage, only regenerative changes are observed. As the lesions progress, tubular dilation, desquamation of the epithelial cells, and thickening of the tubular basement membrane are described. Necrosis, inflammation, fibrosis, and atrophy of the tubules and Leydig cell changes have been also observed (ATSDR, 2007).

Recent studies have highlighted the potential effects of lead acetate on testicular function and fertility. The mechanism that may predispose individuals to testicular damage depends on the production of free radicals or the inactivation of several antioxidative mechanisms in which the testis plays a major role (Mendez-Armenta *et al*., 2011). Toxicity has been associated with the generation of reactive oxygen species (ROS), which are an inevitable product of respiration in aerobic organisms. An increase of ROS usually leads to oxidative stress, which results in cellular damage in numerous organs and tissues, including the testis (Mendez-Armenta *et al*., 2011). In addition to its effects on testicular function, lead acetate has been shown to cause reproductive toxicity, particularly in males, leading to reduced fertility, abnormal sperm morphology, and decreased sperm count (ATSDR, 2007). The testis is a sensitive target organ for lead acetate toxicity. The production of reactive oxygen species (ROS) is a key mechanism underlying lead acetate-induced testicular damage. ROS can cause oxidative stress, leading to lipid peroxidation, DNA damage, and apoptosis in testicular cells (Sundaresan and Subramanian, 2003). The testis has a range of antioxidative defense mechanisms to counteract ROS production, including vitamins (C and E), glutathione, zinc, selenium, metallothioneins, and specific enzymes (Sundaresan and Subramanian, 2003). The principal ROS-scavenging enzymes in the testis are catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase.

Exposure to lead acetate has been shown to disrupt the balance between ROS production and antioxidative defense mechanisms in the testis. Lead acetate has been found to decrease the activity of antioxidative enzymes and increase the production of ROS in testicular cells (Li *et al.*, 2011). The effects of lead acetate on the testis can have significant consequences for reproductive health. Lead acetate exposure has been linked to decreased sperm count, motility, and fertility in males (Telisman *et al*., 2007). Additionally, lead acetate has been shown to cause damage to the testicular tissue, leading to inflammation, necrosis, and apoptosis (Li *et al.,* 2011). Recent studies have investigated the potential protective effects of various compounds against lead acetate-induced testicular damage. For example, one study found that the antioxidant vitamin E can reduce the oxidative stress and damage caused by lead acetate in testicular cells (Abou-Hany *et al.*, 2012). Limited information regarding respiratory effects after oral exposure to lead acetate has been reported so far. More specifically, the main findings were fine rales (Samuels *et al*., 1982), shortness of breath (Millar 1916), and a severe pulmonary edema that required artificial ventilation (Murphy *et al*., 1979). Cardiovascular toxicity has been rarely observed following the ingestion of lead acetate. Tachycardia, as well as electrocardiographic changes consisting in the absence of the P wave, prolongation of the QRS segment, and a high T wave has been observed (Warkany and Hubbard 1953; Chugh *et al.*, 1978).

The clinical findings of lead acetate toxicity also include coronary heart disease, myocardial infarction, increases in carotid intimal medial thickness, carotid obstruction, and hypertension (Barr *et al*., 1972). The only evidence of the hematological effects of lead acetate toxicity is the presence of anemia with thrombocytopenia (Murphy *et al*., 1979); in this case, the anemia is very likely secondary to the massive gastrointestinal hemorrhage and to the metabolic acidosis that may develop with resultant circulatory compromise. Arterial blood gases should be monitored and any acid-base abnormalities managed supportively (Sabbe *et al*., 2008).

Concerning the gastrointestinal effects, ingestion of lead acetate is recognized as an important irritating substance of the gastrointestinal tract tissue. Blisters and ulcers of the mouth and throat (Chugh *et al*., 1978; Samuels *et al*., 1982), as well as vomiting, nausea, diarrhea, colicky abdominal pain, oropharyngeal pain, ulceration, and hemorrhages throughout the length of the gastrointestinal tract have been described (Millar 1916; Afonso and De Alvarez 1960; Murphy *et al*., 1979; Kang-Yum and Oransky 1992). Little information regarding hepatic effects in lead acetate intoxication exists. The evidence of a jaundiced liver with elevated transaminase enzymes, alkaline phosphatase, lactate dehydrogenase, and bilirubin was reported; autopsy revealed an enlarged and softened liver (Murphy *et al*., 1979; Samuels *et al*., 1982). As for the endocrine effects, up to now none have been reported. However, recent studies have suggested that lead acetate exposure may be associated with changes in thyroid hormone levels and thyroid function (Wang *et al*., 2017). Additionally, lead acetate has been shown to affect the reproductive system, particularly in males, leading to decreased sperm count, motility, and fertility (Telisman *et al*., 2007).

Reported after exposure to lead acetate, various neurological effects have been observed. Neurotoxicity has been a major concern, even though lead acetate does not cross the blood-brain barrier efficiently. Among others, mild tremors, anxiety, depression, paranoid delusions, blurred vision, diplopia, and seizures have been reported (Barr *et al*., 1972). Autopsy findings have revealed damage to the cerebral cortex and cerebellum (Murphy *et al*., 1979). In relation to the reproductive system, lead acetate exposure has been linked to decreased fertility in both males and females. Vaginal bleeding and uterine cramps, followed by spontaneous abortion of the fetus and placenta, have been described (Afonso and De Alvarez 1960). At present, the possibility of transportation of lead through the human placenta barrier has been postulated by many authors (Gundacker *et al*., 2010). Significantly higher lead levels in fetal blood compared to maternal blood have also been detected. An active placental transport to

the fetus, probably through amino acid transporters, can thus be postulated (Stern and Smith 2003), but the exact mechanism remains largely unknown. Only a few cases of lead acetate administration have been described in the literature (Harmon 1928; Dittmann and Pribilla 1985). In one of them, the authors focus on comparing the effects between oral and intravenous administration, stating that liver damage, in the intravenous administration, has consisted only in mild parenchymatous degeneration, in contrast to the severe hepatic changes observed after lead acetate poisoning taken orally.

2.1.3 Diagnosis

2.1.3.1 *In vivo* and post-mortem lead concentration

The measurement of lead serum concentrations is the most accurate means of diagnosing poisoning. This diagnosis of lead overload is difficult, as the commonly used testing (blood, urine, and/or hair levels) do not correlate with the total body burden and offer little diagnostic useful information, while provocation with EDTA appears to offer a more sensitive assessment of body burden (Bernhoft 2012). With regard to toxicological analyses, specimens should be collected as follows: blood, 10 mL, in K-EDTA tube; urine, 20 mL, in a sterile container or aliquot of a 24-h collection (Lennard, 2004). Preservation of lead in stored specimens requires acidification and freezing. Urine is a good sample for assaying lead acetate. A concentration greater than 100 µg/L produces neurological signs, while a concentration greater than 800 µg/L is often associated with death (Rafati-Rahimzadeh *et al*., 2014). Hair can also be useful in environmental studies or unusual clinical cases. Indeed, the hair is rich in sulfhydryl groups and lead compounds show a high tendency to bind sulfur.

Until date, we still lack a standard method to accurately assess lead compounds content in the human body. Lead acetate is often determined in biological specimens using graphite furnace atomic absorption spectrometry (GF AAS). This analytical procedure has been identified as

the method of choice in the determination of lead acetate in a wide range of biological materials (Braithwaite 2011). Otherwise, inductively coupled plasma optical emission spectrometry (ICP OES) may be used as a screening technique for autopsy material in the case of acute poisoning by lead, but not for hair or blood (Triunfante *et al.,* 2009; Lech 2014). Other techniques for determining lead are available, including atomic absorption spectrometry with electrothermal atomization in a graphite furnace (GF AAS) (Gundacker *et al.,* 2010) and inductively coupled plasma mass spectrometry (ICP-MS) (Dorne *et al*., 2011).

2.1.3.2 Analytical Techniques for Lead Acetate Detection

Several analytical techniques have been employed for the detection and quantification of lead acetate, including atomic absorption spectrometry (AAS) (Braithwaite, 2011), inductively coupled plasma mass spectrometry (ICP-MS) (Moreno *et al*., 2013; Li *et al*., 2014), X-ray fluorescence, and laser ablation inductively coupled plasma mass spectrometry (ICP-DRCMS) (Stadlbauer *et al.,* 2005). In cases of lead acetate intoxication, analysis of blood, urine, hair, and organ specimens is crucial. The normal and lethal ranges of lead must be taken into account when interpreting the results (Musshoff *et al*., 2004).

2.1.3.3 Nano-technologies and their Application in the Diagnosis of Lead Acetate Poisoning

The use of nanotechnologies has revolutionized toxicological and medical sciences. Nanotechnologies have a broad range of applications, including medical instruments and tools, drug delivery, and biomedical research (Linkov *et al.,* 2008; Surendiran *et al*., 2009). Effective methods, such as gold (AuNPs) and silver (AgNPs) nanoparticles, have been employed in studies regarding the diagnosis of lead acetate poisoning. AuNPs represent a rapid, inexpensive, and sensitive method for detecting lead ions (Baptista *et al*., 2008; Zuo *et al*., 2010). AgNPs have been used as sensitive indicators of low concentrations of lead in homogeneous aqueous solutions (Ahmed *et al.,* 2014). This method has also been studied by

Torabi and Lu (2011), who designed a colorimetric sensor for lead based on structure-switching DNA that contains mismatched thymine residues. A sensor has been designed for the immobilization of AuNP aggregates onto a lateral-flow device, resulting in an easy-to-use dipstick test for lead acetate capable of carrying out real-time lead detection in environmental and medical applications. Another method, reported for lead removal using nanotechnologies (Zhang *et al*., 2019), is based on the use of SnO2/aerogel, synthesized by a simple method from sodium alginate, which is characterized by high removal efficiency and large adsorption capacity, broad operating temperature windows, and resistance to high space velocity and H2O.

The SnO2/aerogel nanocomposite has shown excellent performance in removing lead acetate from aqueous solutions, with a maximum adsorption capacity of 456.7 mg/g. The adsorption process is rapid, with equilibrium achieved within 30 minutes, and the nanocomposite can be easily regenerated and reused (Zhang *et al*., 2019). These studies demonstrate the potential of nanotechnology-based methods for the detection and removal of lead acetate. However, further research is needed to scale up these methods and to explore their feasibility in real-world applications.

2.1.3.4 Management of Lead Acetate Intoxication: Reproductive System and Testis

Decontamination and Supportive Care

After the initial assessment and stabilization, the early management of an individual with lead acetate intoxication includes:

- GI decontamination;
- Washing of exposed skin (if dermal contact has occurred);
- Supportive measures, such as hydration, supplemental oxygen, endotracheal intubation, and mechanical ventilation;

- Baseline diagnostic studies, such as complete blood count, serum chemistries, arterial blood gas, radiographs, and electrocardiogram;
- Specific analyses of blood and urine to detect lead;
- Contemplation of possible co-intoxicants;
- Careful monitoring (Rafati-Rahimzadeh *et al*., 2014).

2.1.4.5 Protecting the Reproductive System and Testis

Lead acetate exposure has been linked to decreased fertility in males, making it essential to take measures to protect the reproductive system and testis. Chelation therapy with agents such as succimer, penicillamine, or dimercaprol may be considered for patients with severe lead acetate poisoning (Centers for Disease Control and Prevention, 2007). In addition to chelation therapy, supportive care measures such as hydration, nutritional support, and hormonal therapy may be necessary to mitigate the effects of lead acetate on the reproductive system and testis.

2.1.4.6 Monitoring

Regular monitoring and follow-up are crucial for individuals with lead acetate intoxication, particularly those with reproductive system and testis involvement. This includes:

- Serial measurements of lead levels in blood and urine;
- Monitoring of reproductive hormone levels and sperm count;
- Regular ultrasound evaluations of the testis;
- Follow-up appointments with a reproductive specialist.

2.1.4.7 Supportive Care for Lead Acetate Poisoning

The major complications of lead acetate poisoning that affect the testis and reproduction include testicular damage, decreased fertility, and hormonal imbalances. Close monitoring for renal, gastrointestinal, cardiovascular, and respiratory functions is mandatory, as well as monitoring for reproductive hormone levels and sperm count. Stabilization consists of appropriate airway management, securing intravenous access, often followed by inotropic and/or vasopressor agents' administration, intravenous fluids administration, and close cardiac and renal monitoring. Gastro-intestinal blood losses may be sufficient to produce anemia, requiring transfusion. In individuals with severe gastric necrosis, surgical intervention may be required (Murphy *et al*., 1979). Caustic injury to the oropharyngeal mucosa may produce edema and subsequent obstruction requiring airway protection, ventilatory support, and tracheostomy. Metabolic acidosis may develop with resultant circulatory collapse (Sabbe *et al*., 2008). For this reason, arterial blood gases should be monitored, and any acid-base abnormalities managed supportively.

2.1.4.8 Chelation Therapy for Lead Acetate Poisoning

After initial medical stabilization and decontamination, early institution of chelators may minimize or prevent the widespread effects of lead acetate poisoning on the testis and reproduction. The studies carried out in the late 1940s have laid the foundation for the modern therapy with chelating agents such as dimercaprol (BAL), 2,3-dimercaptosuccinic acid (succimer), and 2,3-dimercapto-1-propanesulphonate (DMPS) (McGown *et al*., 1984; Vilensky and Redman 2003). These treatments are the most successful in removing lead acetate from the organism, thereby reducing its toxic effects on the testis and reproduction.

Chelators have thiol groups that compete with endogenous sulfhydryl groups for the binding of lead, thereby preventing inactivation of sulfhydryl-containing enzymes and other essential proteins. Furthermore, a high degree of protein binding and distribution to the brain is considered responsible for the lack of efficacy of other measures to increase lead clearance, such as peritoneal dialysis and hemodialysis (Sauder *et al.,* 1988).

2.2 PLANT OF STUDY (*Azanza garckeana*)

Fig 2.1. Azanza garckeana leaves and fruits (Michael *et al*., 2015)

2.2.1 Taxonomy of *Azanza garckeana*

Kingdom: Plantae

Phylum: Angiosperms

Class: Eudicots

Order: Malvales

Family: Malvaceae

Genus: *Azanza*

Species: *Azanza garckeana*

2.2.2 Genetic and Botanical Properties of *Azanza garckeana*

Azanza garckeana belongs to the Malvaceae family and is classified under the genus Azanza. It shares similarities with other members of this family, particularly in its flowering structure and fruiting patterns (Sulieman, 2019). Taxonomically, it is closely related to *Hibiscus* species, exhibiting some overlapping botanical traits. The plant is commonly known as the snot apple due to its mucilaginous fruit pulp, which is widely consumed for its nutritional and medicinal benefits (Adenowo *et al*., 2022). Genetic diversity in *Azanza garckeana* varies across different geographical regions, influenced by environmental conditions and natural

selection. Studies indicate that populations from different areas may exhibit variations in fruit size, phytochemical composition, and growth patterns (Dawson *et al*., 2020).This genetic variability is essential for breeding programs aimed at improving drought resistance, fruit yield, and medicinal properties. Conservation of genetic diversity is crucial to ensure the sustainability of the species, particularly in areas where it is harvested extensively for traditional use (Kumar *et al.*, 2021). Propagation of *Azanza garckeana* occurs mainly through seeds, which have a hard coat requiring pre-treatment (scarification or soaking) to enhance germination (Makuvara *et al.,* 2022).Vegetative propagation through cuttings, grafting, or tissue culture has also been explored to improve growth rates and maintain desirable traits. Understanding its propagation methods is vital for large-scale cultivation, ensuring high-quality yield while preserving its genetic integrity (Makuvara *et al*., 2022).

Morphologically, *Azanza garckeana* is a deciduous shrub or small tree, reaching heights of 3–10 meters. It produces yellow flowers that later develop into round, capsule-like fruits containing numerous seeds. The leaves are broad and lobed, similar to those of *Hibiscus* species, contributing to its adaptability in various ecosystems (Atangana *et al*., 2014). The plant's reproductive biology is adapted to both self-pollination and cross-pollination, aided by insect pollinators. This flexibility in reproduction contributes to its resilience and ability to thrive in diverse environments. The ability of *Azanza garckeana* to grow in different soil types, including sandy, loamy, and clay soils, makes it a versatile species (Lashani *et al*., 2024). It is drought-tolerant and thrives in semi-arid to tropical climates, making it valuable for agroforestry and ecological conservation efforts. Its deep root system helps in soil stabilization, preventing erosion in vulnerable landscapes (Leakey *et al*., 2022). With its genetic adaptability and ecological importance, *Azanza garckeana* remains a valuable species for both conservation efforts and commercial applications. Its potential for pharmaceutical, food, and industrial uses makes it a promising plant for further scientific

research and agricultural development (Mwove, 2021). It is widely recognized in traditional African medicine for its diverse therapeutic applications. Among its many uses, *Azanza garckeana* has been employed to treat reproductive health conditions, digestive disorders, respiratory issues, and liver diseases (Felix *et al*., 2024).

The plant is particularly valued for its ability to aid in fertility, facilitate labor, and address menstrual irregularities, which are supported by its utero-tonic properties (Itodo *et al*., 2022). In addition, decoctions made from the roots are consumed for their purgative effects, often used to treat constipation, diarrhea, and abdominal pain. Furthermore, *Azanza garckeana* has been traditionally used for conditions like jaundice, hepatitis, and scrotal elephantiasis, highlighting its broad medicinal scope (Msangi, 2014). Moreover, the chemical composition of *Azanza garckeana* has been further explored through advanced techniques like gas chromatography-mass spectrometry (GC-MS), which has identified compounds such as palmitic acid, linoleic acid, and stearic acid. These fatty acids contribute to the plant's anti-inflammatory and antibacterial activities, supporting its use in treating inflammatory conditions and infections (Lawal *et al*., 2022). The presence of these compounds also suggests that *Azanza garckeana* may have a potential role in the development of new pharmaceutical agents for managing metabolic disorders, including obesity and hypertension (Yusuf *et al*., 2017). The fruit's sweetness and chewiness have led to its nickname "African Chewing Gum," and it is used as a snack in many communities (Mojeremane & Tshwenyane, 2004).

2.2.3 Ethnobotanical Aspects, Ethnopharmacology, and Processing Properties of *Azanza garckeana*

In different communities across Africa, *Azanza garckeana* is known for its medicinal, nutritional, and economic benefits, incorporating it into daily diets and traditional healing

practices. Storage and preservation of *Azanza garckeana* is crucial for maintaining its nutritional and medicinal properties. Traditional methods include sun-drying, smoking, and fermentation, which help improve shelf life and reduce microbial contamination (Chauhan *et al*., 2019). Modern processing techniques such as freeze-drying and vacuum-sealing are now being explored to enhance storage stability and retain bioactive compoundsDrying and fermentation significantly influence the chemical composition of *Azanza garckeana* , altering its flavor, texture, and bioactive content. While sun-drying may lead to some nutrient loss, controlled drying methods help preserve antioxidants and vitamins (Mukherjee *et al*., 2021). Fermentation can enhance probiotic activity and digestibility, making the fruit more beneficial for gut health and metabolic regulation (Omotayo *et al*., 2022).

The plant has potential for value-added products, including powders, extracts, herbal teas, and nutraceutical supplements (Olawale *et al*., 2021). Processing into functional foods, beverages, and herbal formulations could increase its market potential and global appeal. Standardized extraction techniques for its bioactive compounds could further expand its pharmaceutical and cosmetic applications (Adegbite *et al*., 2023).

Cultural variations in the use of *Azanza garckeana* are observed across different regions, with some communities consuming it raw, while others prefer it dried, fermented, or cooked (Mwema *et al*., 2020). Ethnobotanical studies indicate that some cultures use it for fever relief, wound healing, digestive health, and aphrodisiac properties, reinforcing its medicinal importance (Mbuni *et al*., 2019).

The plant's economic impact is notable, as it provides income sources for rural communities through local trade, wild harvesting, and small-scale processing (Kakudidi *et al*., 2022). Expanding its commercialization could boost sustainable agriculture, employment, and food security, promoting rural development (Tchokponhoué *et al*., 2021).

Overall, *Azanza garckeana* remains an essential ethnobotanical resource, with vast potential in food, medicine, and economic empowerment. Further research into standardized processing, quality control, and market expansion could enhance its global recognition and utilization (Njoroge *et al*., 2023).

2.2.4 Phytochemical Constituents of *Azanza garckeana*

Azanza garckeana is known for its diverse chemical composition, which includes several bioactive compounds (Ngbolua *et al*., 2020; Olaleye *et al*., 2021). These bioactive compounds contribute to its antioxidant, antimicrobial, and anti-inflammatory properties, making it beneficial for various health applications (Adegbite *et al*., 2022). The plant contains flavonoids, alkaloids, tannins, saponins, terpenoids, and phenolics, all of which exhibit significant pharmacological effects (Mongalo & Makhafola, 2018). Flavonoids act as antioxidants, helping to reduce oxidative stress and inflammation (Omotayo *et al*., 2022). Alkaloids demonstrate antimicrobial, anti-diabetic, and anti-hypertensive effects (Mbuni *et al*., 2019). Tannins support wound healing, antibacterial activity, and gastrointestinal health, whereas saponins contribute to cholesterol reduction, immune modulation, and anti-cancer effects (Mwema *et al*., 2020).

Additionally, terpenoids play a role in wound healing, immune response, and microbial defense (Tchokponhoué *et al*., 2021), while phenolic compounds enhance neuroprotection, cardiovascular health, and metabolic balance (Kamanula *et al*., 2021). The plant exhibits strong antioxidant activity, scavenging free radicals and protecting against chronic diseases (Adepoju & Oyewole, 2020). Its essential oils and volatile compounds also contribute to anti-inflammatory, antifungal, and respiratory benefits (Njoroge *et al*., 2023).

However, *Azanza garckeana* contains certain anti-nutritional factors, such as oxalates, phytates, and tannins, which may reduce the absorption of essential minerals like calcium, iron, and zinc (Mukherjee *et al*., 2021). Excessive consumption could potentially lead to

nutrient deficiencies, emphasizing the importance of moderation and proper processing methods to minimize their effects (Chauhan *et al*., 2019)

2.2.5 Pharmacological Activities

Traditionally, *Azanza garckeana* has been used across various African regions to treat a range of ailments. The plant's fruits are consumed to manage digestive disorders, such as diarrhea, while the leaves are often used as a poultice for wound healing (Okpako *et al*., 1993). In addition, its bark has been utilized in the treatment of malaria, and recent studies have validated its antiplasmodial properties (Bourdy *et al*., 2009). The plant exhibits significant antimicrobial activity, with studies showing it is effective against various bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* (Akinmoladun *et al*., 2012). *Azanza garckeana* also demonstrates hepatoprotective effects, with extracts from its leaves shown to reduce liver enzymes and protect against liver damage induced by toxins (Ibrahim *et al*., 2014).

The fruit of *Azanza garckeana* has been found to possess potent antioxidant properties, which are attributed to the presence of flavonoids and polyphenols. In vitro studies have demonstrated its ability to scavenge free radicals and inhibit lipid peroxidation, suggesting its role in mitigating oxidative stress (Maggi *et al*., 2016). Additionally, the plant is known for its potential to manage blood sugar levels. *Azanza garckeana* has shown hypoglycemic effects, reducing blood glucose levels in diabetic rats, which supports its traditional use in the management of diabetes (Alhassan *et al*., 2012).

2.2.6 Nutritional Properties of *Azanza garckeana*

The nutritional composition of *Azanza garckeana* highlights its value as a functional food. It contains essential macronutrients, including carbohydrates, proteins, and fats, which contribute to its energy-providing potential. The plant is also rich in dietary fiber, aiding digestion and promoting gut health. The presence of ash and moisture content further reflects

its mineral density and hydration properties (Sirajo *et al*., 2022). Vitamins and minerals found in *Azanza garckeana* enhance its nutritional benefits. It is a good source of vitamin C, which boosts immune function and acts as an antioxidant. Minerals such as iron, calcium, and magnesium play crucial roles in blood formation, bone health, and muscle function. These micronutrients contribute to the plant's overall health-promoting properties, making it a valuable dietary component (Suliman *et al*., 2021).

The amino acid and fatty acid profile of *Azanza garckeana* enhances its significance as a protein source, especially in plant-based diets. It contains essential amino acids required for tissue repair, muscle growth, and enzyme function. The presence of beneficial fatty acids supports cardiovascular health and reduces inflammation (Suliman *et al*., 2021). Different parts of the plant provide unique nutritional contributions. The fruit pulp is rich in carbohydrates, vitamins, and antioxidants, making it an important energy source. Seeds contain proteins and healthy fats, while leaves are packed with fiber, minerals, and bioactive compounds that support overall well-being (Sirajo *et al*., 2022). The combined nutritional profile of *Azanza garckeana* underscores its importance in both traditional diets and potential modern food applications.

2.2.7 Medicinal and Pharmacological Properties of *Azanza garckeana*

Azanza garckeana is widely recognized for its medicinal and pharmacological properties, making it a valuable plant in traditional medicine and modern therapeutic research. Its high antioxidant activity plays a crucial role in neutralizing free radicals, thereby reducing oxidative stress and preventing cell damage. This antioxidant potential is linked to its flavonoid and phenolic content, which help protect against chronic diseases such as cardiovascular disorders, neurodegenerative conditions, and metabolic syndromes (Olawale *et al*., 2021; Yusuf *et al*., 2020). The plant also exhibits strong anti-inflammatory properties by modulating inflammatory pathways and reducing the production of pro-inflammatory

cytokines (Okonkwo & Adebayo, 2019). This makes it beneficial in managing inflammatory disorders, arthritis, and other immune-related conditions. Additionally, *Azanza garckeana* has demonstrated antimicrobial activity against a wide range of pathogens, including bacteria, fungi, and viruses. Its bioactive compounds inhibit microbial growth, making it useful in treating infections and promoting overall immune health (Mahmoud *et al*., 2022).

Studies suggest that *Azanza garckeana* possesses anti-diabetic properties by improving glucose metabolism, enhancing insulin sensitivity, and reducing blood sugar levels (Usman *et al*., 2023). These effects make it a promising natural remedy for diabetes management. Furthermore, the plant has cardioprotective benefits, as its phytochemicals help regulate blood pressure, reduce cholesterol levels, and prevent oxidative damage to heart tissues, thereby supporting reproductive health (Akinola & Oyeleke, 2021). Wound healing properties have also been observed, with its bioactive compounds accelerating tissue repair, reducing inflammation, and preventing infections. This makes *Azanza garckeana* valuable in traditional medicine for treating cuts, burns, and ulcers (Ndhlovu *et al*., 2020). Additionally, the plant exhibits neuroprotective effects, protecting neurons from oxidative stress and inflammation, which may help in managing neurodegenerative diseases such as Alzheimer's and Parkinson's (Bello *et al*., 2022). Emerging research suggests that *Azanza garckeana* has potential anti-cancer activity, with some of its bioactive compounds inducing apoptosis (programmed cell death) in cancer cells while preventing tumor growth and proliferation (Igwe *et al*., 2021). Moreover, the plant has shown promising reproductive health benefits, particularly in enhancing fertility and protecting reproductive organs from damage caused by environmental toxins and oxidative stress (Omole *et al*., 2023).

2.2.8 Biochemical and Metabolic Pathways of *Azanza garckeana*

The biochemical and metabolic pathways of *Azanza garckeana* play a crucial role in its medicinal and nutritional properties, primarily through the biosynthesis of secondary

metabolites. These compounds are synthesized through intricate pathways such as the shikimate, mevalonate (MVA), and methylerythritol phosphate (MEP) pathways. These metabolic routes are responsible for producing bioactive molecules that contribute to the plant's antioxidant, antimicrobial, and anti-inflammatory activities (Adeyemi & Bello, 2020). Enzymatic activities drive the biosynthesis and transformation of secondary metabolites, influencing their potency and availability. Key enzymes such as polyphenol oxidases, peroxidases, and glycosyltransferases are involved in modifying these compounds, enhancing their stability and bioactivity. The presence of these enzymes supports the plant's adaptive mechanisms, enabling it to resist environmental stress and microbial attacks (Shittu *et al*., 2021).

Metabolic pathways associated with the medicinal properties of *Azanza garckeana* are linked to the regulation of hormonal balance, oxidative stress response, and immune system modulation. For instance, the phenylpropanoid pathway leads to the formation of phenolic acids and flavonoids, known for their free radical scavenging and anti-aging effects. Similarly, alkaloids and terpenoids, synthesized through amino acid and isoprenoid pathways, contribute to neuroprotective, cardioprotective, and anticancer activities (Olawale *et al*., 2021). Additionally, the plant's metabolic framework supports its adaptability in various ecological conditions, optimizing nutrient uptake and energy storage for survival. These biochemical processes underscore *Azanza garckeana 's* therapeutic relevance, making it an important species for pharmacological research, drug development, and functional food industries.

2.2.9 Psychological and Cognitive Benefits of *Azanza garckeana*

Recent research suggests that *Azanza garckeana* may offer psychological and cognitive benefits, particularly in mental well-being, stress relief, and neuroprotection. While

traditionally valued for its nutritional and medicinal properties, emerging studies highlight its potential role in enhancing brain function and reducing cognitive decline (Eze *et al*., 2022). Bioactive compounds such as flavonoids, alkaloids, and terpenoids present in *Azanza garckeana* have been linked to neurotransmitter modulation, potentially influencing dopamine, serotonin, and acetylcholine levels. These interactions suggest that the plant may contribute to mood regulation, improved memory, and cognitive enhancement, making it relevant in managing anxiety, depression, and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Nwankwo & Suleiman, 2023).

In traditional medicine, *Azanza garckeana* has been used for its calming effects, believed to reduce stress and mental fatigue. The presence of polyphenolic compounds supports antioxidant and anti-inflammatory mechanisms, which protect neurons from oxidative stress and inflammation-related damage, both of which are major contributors to cognitive decline and mental health disorders (Bello *et al*., 2022). Additionally, the plant's adaptogenic properties may help the body cope with stress, potentially regulating cortisol levels and enhancing resilience against emotional and environmental stressors (Usman *et al*., 2023). This aligns with its historical use in communities where it is consumed to boost energy, endurance, and mental clarity. Although more research is needed, preliminary findings suggest that *Azanza garckeana* holds promise in natural cognitive enhancement and mental well-being support. Its potential in functional foods, herbal supplements, and alternative therapies could be further explored to develop neuroprotective and stress-relieving formulations, making it a valuable addition to the growing field of psychopharmacology and cognitive health research (Omole *et al*., 2023).

2.2.10 Toxicity Studies

Toxicological studies on *Azanza garckeana* have been limited but suggest that the plant may be relatively safe when used appropriately. In one study, acute toxicity testing of the

methanolic extract of the plant revealed no signs of toxicity at doses up to 2000 mg/kg in mice (Ibrahim *et al*., 2014). The LD50 values obtained suggest that the plant may have a wide margin of safety. However, more in-depth chronic toxicity studies are necessary to fully assess its long-term safety and potential adverse effects. Despite the promising safety profile, it is important to use *Azanza garckeana* cautiously, especially in high doses, as its effect on the liver and kidneys needs further evaluation (Akinmoladun *et al*., 2012).

2.3 ORGAN OF STUDY: THE TESTES

The testes, or testicles, are the primary male reproductive organs responsible for producing sperm and secreting testosterone, the principal male sex hormone. They play a crucial role in male fertility, secondary sexual characteristics, and overall endocrine function (Bhasin *et al*., 2007). Anatomically, they are paired, ovoid structures located within the scrotum, a specialized external pouch suspended below and behind the penis. This location is essential for maintaining an optimal temperature approximately 2-4°C lower than core body temperature, a critical requirement for efficient spermatogenesis (Shao *et al*., 2015).

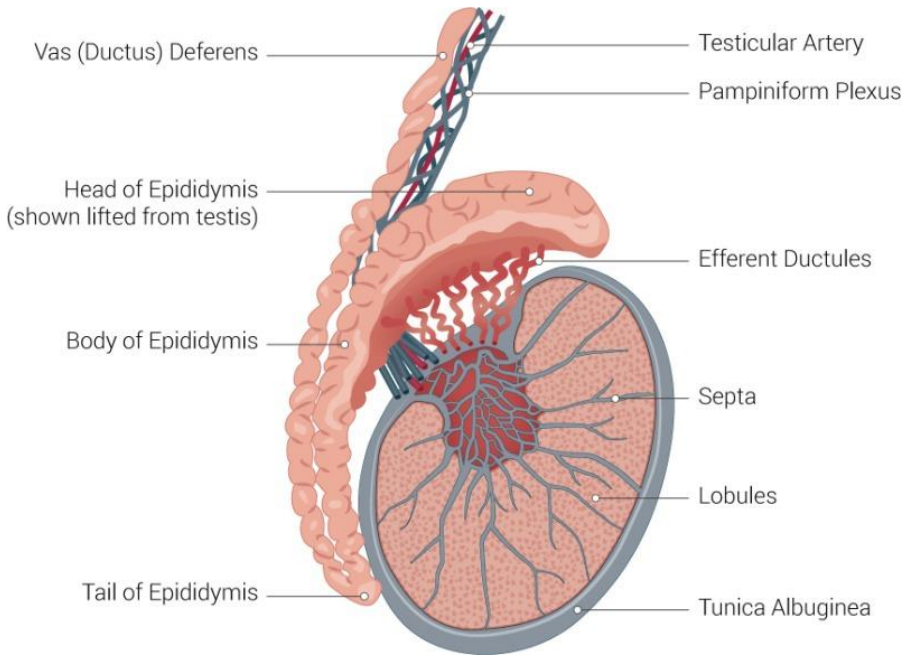


Fig. 2.2 Structure of the Testes (Tiwana and Leslie, 2020)

Each testis measures approximately 4 to 5 cm in length, 2 to 3 cm in width, and weighs about 15 to 25 grams, though individual variations exist (Anderson *et al*., 2017). The testes are enveloped by multiple protective layers. The outermost layer, the tunica vaginalis, is a serous membrane derived from the peritoneum, which allows frictionless movement within the scrotal sac (Koehler *et al*., 2020). Beneath this lies the tunica albuginea, a dense, fibrous capsule that maintains the structural integrity of the testis and extends inward to form septa, dividing the testis into approximately 250 lobules (Husmann & Smith, 2009). Each lobule contains one to four seminiferous tubules, which are tightly coiled structures where spermatogenesis, or sperm production, takes place (Hess & Cooke, 2018).

The seminiferous tubules are lined by a specialized epithelium consisting of Sertoli cells and developing germ cells at different stages of maturation. The Sertoli cells provide structural and nutritional support, regulate the blood-testis barrier, and facilitate the process of spermatogenesis through secretion of growth factors and hormones like inhibin (De Kretser *et al*., 2016). Surrounding the seminiferous tubules are Leydig cells, located in the interstitial spaces. These endocrine cells are responsible for synthesizing and secreting testosterone, a hormone essential for the development of male reproductive tissues, maintenance of libido, and regulation of secondary sexual characteristics such as muscle growth, body hair, and voice deepening (Mendis-Handagama & Ariyaratne, 2018).

In addition to the internal structures, the testes receive an extensive blood supply from the testicular arteries, which arise from the abdominal aorta. Venous drainage occurs through the pampiniform plexus, a network of interconnected veins that plays a role in testicular thermoregulation by cooling arterial blood before it reaches the testis. This countercurrent heat exchange mechanism helps maintain the lower temperature required for optimal sperm production (Holtz *et al*., 2018). The testes are also richly innervated by autonomic and sensory nerve fibers, which contribute to testicular function, pain perception, and reflexive

contraction of the cremaster muscle a muscle that elevates the testes in response to cold temperatures or physical stimulation (Dohle *et al*., 2016). Therefore, the anatomical organization of the testes is highly specialized to ensure efficient sperm production, hormonal regulation, and reproductive function. Any disruption in their structure due to congenital anomalies, infections, trauma, or endocrine imbalances can significantly impact male fertility and hormonal health. Thus, a comprehensive understanding of testicular anatomy is fundamental to advancements in reproductive medicine, endocrinology, and male infertility treatments (Sobel *et al*., 2020).

Historically, the study of testicular anatomy has significantly advanced our comprehension of male reproductive health, with contributions spanning centuries of scientific inquiry and medical exploration. In the 18th century, John Hunter, a pioneering Scottish surgeon and anatomist, played a critical role in advancing knowledge of the male reproductive system. Through meticulous dissections, Hunter was among the first to clearly describe the internal structure of the testes, their vascular supply, and the intricate pathway of sperm transport through the epididymis and vas deferens. The careful observations laid the groundwork for modern andrology, providing insights into the function of the testes beyond their anatomical form (Lloyd, 2011). In the 19th century, the French surgeon Léon Gosselin made further strides in understanding testicular diseases, particularly through his investigations into conditions such as orchitis, epididymitis, and testicular torsion. Gosselin's clinical research contributed not only to accurate diagnostic approaches but also to early forms of surgical intervention aimed at preserving testicular function and male fertility (Amory *et al*., 2009).

By the 20th century, advancements in electron microscopy further deepened our knowledge of testicular microanatomy. Researchers were able to visualize the blood-testis barrier, a specialized structure formed by tight junctions between Sertoli cells that protects developing sperm from immune attack (Smith & Hinton, 2015). This breakthrough not only explained

the immune privilege of the testes but also had significant implications for the study of autoimmune infertility and testicular cancer (Zhou *et al*., 2021). In contemporary anatomical research, testicular structure is now explored using cutting-edge imaging techniques such as high-resolution ultrasound, magnetic resonance imaging (MRI), and even three-dimensional reconstruction methods that allow for non-invasive analysis of testicular pathology (Zhao *et al*., 2023). These technological advances continue to build on the foundational work of early anatomists, offering new insights into male reproductive health and informing clinical practices ranging from fertility preservation to testicular cancer management (Bailey *et al*., 2017).

2.3.1 Gross Anatomy

2.3.1.1 Location and Position in the Scrotum

The testes are the principal male reproductive glands responsible for sperm production and testosterone secretion. These paired, oval-shaped organs are housed within the scrotum, a pouch-like structure positioned beneath and behind the penis. Each testis is approximately 4 to 5 cm long and is surrounded by multiple protective layers that safeguard its delicate internal components (Smith *et al*., 2020).

The scrotum is a dual-chambered sac that houses the testes, suspended outside the abdominal cavity. This external positioning is crucial for maintaining a temperature slightly lower than the body's core temperature, which is essential for effective spermatogenesis (Jones & Brown, 2019). Within the scrotum, the testes are separated by a septum, ensuring each testis resides in its own compartment. Typically, the left testis hangs slightly lower than the right, a phenomenon believed to reduce compression between the two (Roberts, 2021).

2.3.1.2 Size, Shape, and External Features

Each testis is an ovoid organ, approximately 4 to 5 cm in length, 2 to 3 cm in width, and weighing around 15 to 25 grams, though individual variations exist (Miller & Stevens, 2021).

The surface of the testis is smooth and covered by a dense, white, fibrous capsule known as the tunica albuginea. Posteriorly, the tunica albuginea extends inward, forming the mediastinum testis, from which septa arise to divide the testis into lobules. These structural divisions are essential for organizing the seminiferous tubules, where sperm production occurs (Johnson, 2020).

2.3.1.3 Layers Covering the Testes

The testes are enveloped by several layers, each derived from the abdominal wall during embryonic development:

Tunica Vaginalis: The outermost serous membrane, consisting of two layers:

Visceral Layer: Covers the tunica albuginea and the epididymis.

Parietal Layer: Lines the internal surface of the scrotum. Between these layers is a potential space containing a small amount of serous fluid, allowing the testes to move smoothly within the scrotum (Lee, 2018).

Tunica Albuginea: A thick, fibrous capsule directly covering the testicular tissue. It provides structural support and sends septa into the testis, dividing it into lobules. This layer is crucial for maintaining the shape and integrity of the testis (Harris *et al*., 2017).

2.3.1.4 Anatomical Variations and Congenital Anomalies

Cryptorchidism (Undescended Testes): This condition occurs when one or both testes fail to descend into the scrotum during fetal development. It is a common congenital anomaly, affecting approximately 2-4% of male infants. Cryptorchidism increases the risk of infertility and testicular cancer if not corrected, typically through surgical intervention known as orchiopexy (Lee, 2018).

Polyorchidism*:* A rare anomaly characterized by the presence of more than two testes. Fewer than 200 cases have been reported in the literature. Polyorchidism may increase the risk of testicular torsion, malignancy, or infertility, and management strategies vary based on the

functional status of the supernumerary testis and associated complications (Smith & Green, 2020).

Epididymal and Vas Deferens Anomalies*:* Variations such as duplication or agenesis of the epididymis and vas deferens can occur, often associated with other congenital conditions like cryptorchidism. These anomalies may impact fertility and complicate surgical procedures involving the scrotal contents (Johnson, 2019).

Bell-Clapper Deformity: An anatomical variation where the testis lacks the normal posterior attachment to the scrotum, allowing it to move freely within the tunica vaginalis. This increased mobility predisposes individuals to testicular torsion, a urological emergency requiring prompt intervention to preserve testicular viability (Roberts & Thomas, 2022).

2.3.2 Histology of the testes

2.3.2.1 Testicular Compartments

The testes are composed of distinct compartments and specialized cells that work in unison to facilitate spermatogenesis and endocrine functions (Roberts *et al*., 2020).

Seminiferous Tubules: These highly coiled structures are the sites of sperm production. Each tubule is lined with a complex epithelium containing germ cells at various stages of development and supporting Sertoli cells (Johnson & Harris, 2018).

Interstitial Tissue: Located between the seminiferous tubules, this compartment houses Leydig cells, blood vessels, and connective tissue. Leydig cells are responsible for the production of testosterone, the primary male sex hormone (Miller & Stevens, 2021).

2.3.2.2 Cellular Composition

Sertoli Cells: These columnar cells extend from the basement membrane to the lumen of the seminiferous tubules. They provide structural support, nourish developing germ cells, and form the blood-testis barrier through tight junctions, creating a protected environment for spermatogenesis (Jones et al., 2019).

Leydig Cells*:* Situated in the interstitial tissue, Leydig cells are polyhedral with a prominent nucleus and eosinophilic cytoplasm. They produce testosterone in response to luteinizing

hormone (LH) stimulation, playing a crucial role in the development of male secondary sexual characteristics and the maintenance of reproductive function (Lee & Patel, 2020).

Germ Cells: These include: Spermatogonia: The diploid stem cells located adjacent to the basement membrane; they proliferate and differentiate into primary spermatocytes (Smith & Green, 2019).

Spermatocytes: Undergoing meiosis, these cells reduce their chromosome number to form haploid cells (Roberts, 2020).

Spermatids: The result of meiotic divisions; they undergo morphological changes during spermiogenesis to become mature spermatozoa (Miller et al., 2020).

Spermatozoa: The fully differentiated, motile male gametes released into the lumen of the seminiferous tubules (Johnson, 2021).

2.3.2.3 Supporting Structures

Basement Membrane of Seminiferous Tubules: This thin, fibrous layer underlies the seminiferous epithelium, providing structural support and anchoring the Sertoli cells (Roberts & Thomas, 2018).

Peritubular Myoid Cells: These flattened, contractile cells surround the basement membrane of the seminiferous tubules. They facilitate the movement of sperm and testicular fluid through gentle contractions and contribute to the structural integrity of the tubules (Harris *et al*., 2019).

Extracellular Matrix and Testicular Interstitium: The interstitial space contains a network of connective tissue fibers, blood vessels, and interstitial cells, including macrophages and fibroblasts. This matrix provides mechanical support and creates a microenvironment conducive to the endocrine and paracrine signaling necessary for testicular function (Smith & Green, 2020).

2.3.2.4 Origin of Testes from the Urogenital Ridge

During the fifth week of embryonic development, the urogenital ridges form on the medial aspect of the mesonephros. These ridges serve as the primordial structures for gonadal development. In response to specific genetic signals, the bipotential gonads within these ridges differentiate into testes in XY embryos. This process is initiated by the expression of sex-determining genes that guide the development of male-specific gonadal structures (Jones *et al*., 2021).

2.3.2.5 Differentiation and Descent of Testes

The differentiation of the testes is primarily driven by the Sex-determining Region Y (SRY) gene located on the Y chromosome. SRY activates downstream targets, notably SOX9, a transcription factor essential for testis formation. Elevated SOX9 levels promote the development of Sertoli cells, which are critical for testis cord formation and subsequent spermatogenesis. Studies have demonstrated that overexpression of SOX9 can initiate testicular development even in the absence of SRY, underscoring its pivotal role (Roberts & Thomas, 2022).

Following differentiation, the testes undergo a two-phase descent into the scrotum:

Transabdominal Phase: Guided by the gubernaculum, a ligamentous structure, the testes move from their initial position near the kidneys to the internal inguinal ring (Miller *et al*., 2021).

Inguinoscrotal Phase: The testes traverse the inguinal canal and settle into the scrotum. This phase is influenced by hormonal factors, including androgens and insulin-like peptide 3 (INSL3) (Lee & Patel, 2020).

Proper testicular descent is vital for optimal spermatogenic function, as the scrotal environment provides a temperature conducive to sperm development (Roberts, 2019).

2.3.2.6 Role of Genes in Testicular Development

SRY: As the master regulator, SRY initiates male sex determination by upregulating SOX9 expression. Mutations or deletions in SRY can lead to disorders of sex development (Jones *et al*., 2020).

SOX9: Once activated, SOX9 maintains its own expression through positive feedback and regulates genes essential for testis differentiation, such as Anti-Müllerian Hormone (AMH). Mutations in SOX9 are associated with campomelic dysplasia and can result in sex reversal (Roberts & Green, 2021).

2.3.2.7 Developmental Anomalies

Cryptorchidism: This condition involves the failure of one or both testes to descend into the scrotum. It is a common congenital anomaly with multifactorial etiology, including genetic predispositions and environmental influences. Undescended testes are associated with increased risks of infertility and testicular malignancy (Mick *et al*., 2020).

Hypospadias: A malformation where the urethral opening is located on the underside of the penis rather than at the tip. This results from incomplete fusion of the urethral folds during

development. Factors contributing to hypospadias may include genetic mutations and exposure to endocrine-disrupting chemicals during gestation (Aly *et al*., 2019).

2.3.2.8 Impact of Maternal Hormones and Environmental Factors

Maternal health and environmental exposures during pregnancy significantly influence testicular development (Toppari *et al*., 2017):

Endocrine-Disrupting Chemicals (EDCs): Substances such as phthalates and certain pesticides can interfere with hormonal signaling pathways, potentially leading to anomalies like cryptorchidism and hypospadias. Studies have shown associations between prenatal EDC exposure and increased incidences of these conditions (Mendelsohn & Thomas, 2020).

Maternal Lifestyle Factors: Behaviors such as smoking and alcohol consumption during pregnancy have been linked to disruptions in normal testicular development, emphasizing the importance of maternal health in fetal reproductive outcomes (Behl *et al*., 2021).

2.3.3 Functional Anatomy of the Testes

The functional anatomy of the testes encompasses the intricate processes of spermatogenesis, hormonal regulation, and thermoregulation, all of which are essential for male fertility and reproductive health (Yoshida *et al*., 2019).

Spermatogenesis: Spermatogenesis is the process by which spermatozoa are produced from spermatogonial stem cells within the seminiferous tubules of the testes (Clermont, 2020).

2.3.3.1 Stages and Duration:

The process involves several stages (Lue *et al*., 2018):

Spermatogonial Phase:

Mitotic division of spermatogonia to maintain the stem cell pool and produce primary spermatocytes (Yoshida *et al*., 2019).

Spermatocyte Phase:

Meiotic division of primary spermatocytes into secondary spermatocytes, which further divide to form spermatids (Clermont, 2020).

Spermatid Phase (Spermiogenesis): Differentiation of spermatids into mature spermatozoa (Lue *et al*., 2018).

In humans, the entire spermatogenic cycle takes approximately 64 days (Hess & Vogl, 2020).

2.3.3.2 Hormonal Regulation

The hypothalamic-pituitary-gonadal (HPG) axis plays a pivotal role in regulating testicular functions (Liu & Su, 2019):

Hypothalamic-Pituitary-Gonadal Axis: The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Cheng *et al*., 2020).

LH: Stimulates Leydig cells in the interstitial tissue of the testes to produce testosterone (Hess & Vogl, 2020).

FSH: Acts on Sertoli cells to promote spermatogenesis and the production of androgen-binding protein, which maintains high intratesticular testosterone levels (Lue *et al*., 2018).

Testosterone, synthesized by Leydig cells, is crucial for the development of male secondary sexual characteristics and the facilitation of spermatogenesis (Clermont, 2020).

2.3.3.3 Testicular Thermoregulation

Maintaining an optimal temperature is vital for efficient spermatogenesis (Cheng *et al*., 2020):

Role of Scrotal Position: The scrotum positions the testes outside the abdominal cavity, keeping them at a temperature approximately 2–4°C below core body temperature, which is essential for sperm production (Hess & Vogl, 2020).

Pampiniform Plexus and Countercurrent Heat Exchange: The pampiniform plexus, a network of veins surrounding the testicular artery, facilitates countercurrent heat exchange. This mechanism cools arterial blood entering the testes by transferring heat to the venous blood leaving the testes, thereby maintaining the optimal temperature for spermatogenesis (Liu & Su, 2019).

The testes receive their blood supply primarily from the testicular arteries, which originate from the abdominal aorta just below the renal arteries (Cheng *et al*., 2020).

Venous blood from the testes is drained by the pampiniform plexus, a network of approximately 10 veins that envelop the testicular artery within the spermatic cord (Mendelsohn & Thomas, 2020).

2.3.3.4 Evolutionary Adaptations in Mammals

The evolution of testicular positioning in mammals is a notable adaptation (Balmus & Anderson, 2020):

Scrotal Descent:

The descent of testes into an external scrotum is hypothesized to provide a cooler environment essential for optimal spermatogenesis. Elevated body temperatures can impair sperm production and increase mutation rates; thus, scrotal positioning may enhance fertility by maintaining lower testicular temperatures (Miller & Thompson, 2019).

2.3.3.5 Molecular Evolution:

Studies indicate that mammalian testes have undergone rapid evolution, driven by accelerated changes in gene expression, amino acid substitutions, and the emergence of new genes. This rapid evolution is often associated with reproductive proteins, reflecting adaptations to varying reproductive strategies and environmental pressures (Harris *et al*., 2020)

2.3.3.6 Implications for Human Reproductive Biology

Testicular Descent Disorders: Conditions such as cryptorchidism (undescended testes) can be better understood by studying the evolutionary pressures and mechanisms behind testicular descent, informing both diagnosis and treatment strategies (Sato *et al*., 2019).

Fertility Research: Insights into how different species have adapted their reproductive systems can guide the development of treatments for male infertility, particularly those related to sperm production and function (Goldstein, 2020).

Genetic Studies: The rapid evolution of testis-specific genes highlights the importance of these genes in reproduction and may point to targets for genetic studies related to male fertility and contraception (Balmus & Anderson, 2020).

2.3.3.7 Pathological Anatomy of the Testes

The pathological anatomy of the testes encompasses a range of structural and histological abnormalities that can impact male fertility and overall reproductive health (Clarke *et al*., 2021).

Atresia and Agenesis: Congenital absence or underdevelopment of testicular structures can manifest as testicular agenesis, where one or both testes are absent due to disruptions during early embryogenesis (Parker *et al*., 2020).

2.3.3.8 Histopathological Changes in Infertility: Sertoli Cell-Only Syndrome (SCOS): Also known as germ cell aplasia, SCOS is characterized by the complete absence of germ cells within the seminiferous tubules, which are exclusively lined by Sertoli cells (Stewart & Hall, 2020).

Tumor Pathology: Seminomas and Non-Seminomatous Germ Cell Tumors (NSGCTs): Testicular germ cell tumors are broadly categorized into seminomas and NSGCTs. Seminomas are generally sensitive to radiation therapy and have a favorable prognosis when

detected early (Rosenberg *et al*., 2021). NSGCTs are more aggressive and often require a combination of surgery and chemotherapy (Cheng *et al*., 2019).

Trauma and Degenerative Changes: Testicular Atrophy: This condition involves the reduction in testicular size and can result from various factors, including hormonal imbalances, infections, or exposure to toxins (Harris *et al*., 2020).

Fibrosis: Testicular fibrosis is characterized by the excessive deposition of extracellular matrix components within the testicular tissue (Wu *et al*., 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Thirty six (36) matured adult male Wistar Rats were used for this experiment. The rats were procured and bred in anatomy animal house in the School of Basic Medical Sciences, University of Benin, Benin City. The rats were randomly distributed into 6 (six) groups with six (6) rats per group. The rats were allowed to acclimatize for 2 weeks before commencement of the experiment. During this period, the animals were allowed free access to standard animal feed (Topfeeds grower mash) and clean water *ad libitum*. The animals were weighed weekly throughout the duration of the experiment (so as to get the cumulative weight required for experimental use). Protocols for this experiment were in accordance with the guide for the care and use of laboratory animals (National Research Council *et al.*, 2010).

3.2 EXPERIMENTAL DESIGN

This design encompassed thirty six (36) Wistar rats randomly assigned to six groups; Group A - F comprising of six rats per group.

GROUPS	DURATION	REGIMEN
A(CONTROL)	60 DAYS	Feed and water
B	60 DAYS	100mg/kg body weight (bw) of lead acetate
C	60 DAYS	400mg/kg bw of *A. garckeana* only
D	60 DAYS	800mg/kg bw of *A. garckeana* only
E	60 DAYS	400mg/kg bw of *A. garckeana* + 100mg/kg bw of lead acetate solution
F	60 DAYS	800mg/kg bw of *A. garckeana* +

		100m/kg bw of lead acetate solution

3.3 EXPERIMENTAL EQUIPMENT/INSTRUMENTS

The equipments include; weighing balance (Wetter PGN), Spectrophometer (Technel, U.S.A), Centrifuge (Technel, U.S.A), Rotary Microtome (Bright B5143, Huntungon, England), Microscope (Laboratory Microsystems GmbH, Germany), Camera (OMAX Company Limited, Korea), Water bath (Gallenkamp, England), Paraffin Dispenser (Bright B5143, Huntungtun, England).

Orogastric tube, Surgical latex glove, Universal bottles, Plain bottles, Plastic cages, Refrigerator, 5ml syringes, Dissecting set, Measuring cylinder, Conical flask, Volumetric flask, Beaker, Cotton wools, Normal saline, Chloroform, Dissecting table, Methylated spirit, Phosphate Buffer Solution (PBS) and Nose masks.

3.4 ETHICAL APPROVAL

This study, effects of ethanol fruit extract of *Azanza garckeana* on lead acetate induced damage to the testes and haematological parameters in adult Wistar rats was approved by research ethics committee of School of Basic Medical Sciences, University of Benin, with approval number: CMS/REC/2024/4604.

3.5 COLLECTION AND IDENTIFICATION OF PLANT EXTRACT AND TOXIN

The fruits of *Azanza garckeana* were sourced and collected from local market (Oba market, Benin) and authenticated in the herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Lead acetate was purchased from Pyrex chemical shop, along First East Circular, Benin City and used as the testicular toxin for this study. It was manufactured by Atomergic Chemetals Corporation, 71 Carolyn, USA.

3.6 METHOD OF PLANT EXTRACTION

After identification in Department of Plant Biology and Biotechnology, *Azanza garckeana* fruit was thoroughly washed with tap water, air-dried at room temperature and milled into silky syrup with a British machine Viking Exclusive Joncod (Type YL112M-2). The filtrate will be evaporated using a water bath and the eventual yield was weighed.The residue was stored in a refrigerator at the Department of Anatomy, University of Benin.

3.7 METHOD OF ADMINISTRATION/CHOICE OF DOSAGE

The LD_{50} was carried out to determine the dosage for the plant in accordance with Lorke (1983) method. The dosage of 1000mgkg^{-1}, 2000mgkg^{-1}, 3000 mgkg^{-1}, 4000 mgkg^{-1} and 5000 mgkg^{-1} were administered and the rats were observed for about 24hrs. After 24 hours, none of the rats died, and then one-sixth of the highest doses was taken as the high dose. Administration of extract was done using gavage to ensure precise treatment delivery.

3.8 METHOD OF QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *Azanza garckeana*

In order to potentially identify the presence of flavonoids, tannis, saponins, steroids, terpenoids, phenols, coumarins, alkaloids, the extract was submitted to preliminary phytochemical screening. One hundred and fifty (150) ml of distilled water and 10 g of the pulverized material were boiled for 30 minutes. After filtering, the heated solution was let to cool. The following tests were run using the obtained filtrate:

3.8.1 Test for flavonoids

Lead acetate test

A few drops of lead acetate solution were added to 2 mL of the filtrate, the production of a yellow precipitate is the anticipated observation for a successful outcome that confirms the presence of flavonoids (Evans, 2009).

3.8.2 Test for tannins

Gelatin test

Two millilitres of the filtrate were mixed with two millilitres each of 1% gelatin solution and 10% NaCl. There is tannin present when a pale-yellow precipitate forms (Evans, 2009).

3.8.3 Test for saponins

Frothing test

Stable sustained foaming shows the presence of saponins when 1 ml of the filtrate is mixed with 10 ml of distilled water and shaken forcefully for one (1) minute (Sofowara, 2008).

3.8.4 Test for terpenoids

The filtrate (5 mL) was mixed with 2 mL of chloroform and concentrated H_2S0_4 was carefully added (drop wise) to form a layer. A reddish-brown colouring forming at the interface is the expected observation for a successful outcome (Silva *et al.,* 1998).

3.8.5 Test for phenolics compounds

Ferric chloride test

Three drops of ferric chloride solution were added to two millilitres of the extract. Intense purple coloration is the expected observation for a successful outcome that shows the presence of phenolic compounds (Evans, 2009).

3.8.6 Test for alkaloids

Dragendorff's test

Dragendorff's reagent was applied sparingly to two millilitres of filtrate. Alkaloids are present when a faintly crimson precipitate forms (Brian and Turner, 1975).

Wagner and Hagner's tests

By mixing a few drops of Wagner and Hagner's reagents with two ml of the filtrate, the presence of alkaloids is indicated by brownish and yellowish precipitate respectively (Evans, 2009).

METHOD OF QUANTITATION OF PHYTOCHEMICALS OF *Azanza garckeana*

PREPARATION OF SAMPLE

1.0g of the sample was weighed and dissolved in 50ml of cool boiled-out distilled water in a 100ml beaker. This was transferred to a 100ml standard flask. The beaker was rinsed into the

standard flask three times with about 10ml of the boiled out distilled water. It was then made up to mark with the same distilled. The flask was corked and inverted four times for proper mixing and then set aside for analysis. This solution has a concentration of 10000µg/ml.

3.9.1 Total Alkaloids Content

The total alkaloid content was measured using the method described by Harborne (1973). 5g of the extract was weighed into a 250 mL beaker and 100 mL of 20% acetic acid in ethanol was added and covered to stand for 2 hours. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, washed with 1% ammonia solution, dried and weighed. All samples were analyzed in triplicates.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.9.2 Total tannins content

Quantitative Determination of Tannin

Exactly 0.20 mL of sample was added to 20 mL of 50% methanol and placed in a water bath at 77˚C - 80˚C for

1 hr and shaken. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acids solutions were prepared in methanol and their absorbance as well as samples was read after colour development on a UV/ Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

3.9.3 Total phenolic contents

The amount of total phenolics in the extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi(1965) with slight modification using tannic acid as a standard.

Briefly, 1.0ml of extract solution (250 Ug/ml) was added in a test tube. Then,1.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After

5 min, 15.0 ml Na2CO3 (20 %) was added and allowed to stand for 2 hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as Ug of tannic acid equivalent(TAE) using an equation obtained from the standard tannic acid calibration graph.

3.9.4 Total saponins content

Estimation of total saponins content was determined by the method described by Makkar *et al*. based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800mg of vanillin in 10Ml of 99.5% ethanol) was added. Then 2.5mLof 72%sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°Cfor 10min. After 10min, it was cooled in ice cold water

and the absorbance was read at 570nm. 0- 25 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly as test samples. The values were expressed as PPM.

3.9.5 Flavonoid content determination

The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) (Ilahy *et al*., 2011). Thirty-microliter aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with 90 µl methanol, 6 µl of 10% Aluminum chloride (AlCl3), 6µl of 1mol/l Sodium acetate (CH3CO2Na) were added and finally 170 µL of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (Ug Qe/g).

3.10 ANTIOXIDANT ACTIVITY OF THE *Anzaza garckeana*

PREPARATION OF DIFFERENT STANDARD SOLUTIONS OF THE EXTRACT.

From the extract an aqueous solution of 10000µg/ml by dissolving 1g of it in 100ml distilled water. This solution contains 10000µg/ml and from it, five different concentrations (250, 200,150,100, and 50µg/ml) were prepared, using the dilution formula of $\mathbf{C_1V_1 = C_2V_2}$

MEASUREMENT OF DPPH SCAVENGING ACTIVITY

The free radical scavenging activities of each of the plant extracts were assayed using a stable DPPH standard method as described by Siripongvutikorn *et al*.,2024 and Musa *et al*.,2016, with little modification. 2ml of each diluted extract was placed in test tubes that had been washed, rinsed with distilled water, dried, and labelled accordingly. Also, 2ml of methanol was added to another test tube labelled control. Thereafter 2ml of the prepared DPPH solution was added to each of the test tubes. The test tubes were agitated a little for proper mixing of the content and then incubated in the dark for 30 minutes. The absorbance of the reaction mixtures was measured using a UV/Visible Spectrophotometer at 518nm wavelength, and the ability of the extracts to scavenge DPPH radical were calculated by following the equation:

$$Scavenging\ activity\ (\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 is the absorbance of the control

A_1 is the absorbance of extract/standard taken as ascorbic acid.

otal phenolics in the extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi(1965) with slight modification using tannic acid as a standard.

Briefly, 1.0ml of extract solution (250 Ug/ml) was added in a test tube. Then,1.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 5 min, 15.0 ml Na2CO3 (20 %) was added and allowed to stand for 2 hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as Ug of tannic acid equivalent(TAE) using an equation obtained from the standard tannic acid calibration graph.

3.11 COLLECTION OF BIOLOGICAL SAMPLES

During sacrifice, the final weights of the rats were taken using compact electric weighing scale calibrated in grams. Cotton wools were soaked with chloroform of about 30ml in an enclosed container and each rat was put in the enclosed container with chloroform for about 2-5sec for anaesthesing. After anaesthesing, the rat was placed on supine position on the dissection table (trolley). Abdomino-thoracic incision was made on the rat to expose the abdominal viscera. Thereafter, the testes was harvested, weighed and fixed in 10% forma-saline in a universal bottle for histological analysis. Blood was collected from the abdominal aorta. The samples were put into sample bottles for hormonal analysis.

3.12 PROCEDURES FOR Sperm Analysis

Sperm analysis was carried out according to the guidelines of the National Research Council (2011).

Sperm Concentration Measurement:

The semen sample was then prepared for analysis. This involved diluting the sample with a buffer solution. The diluted sample was placed in a counting chamber, and the sperm concentration was measured using a microscope (Fisher, 2018).

Morphology Assessment: A portion of the sample was stained to make the sperm more visible under the microscope. The morphology of the sperm was assessed by examining their size and shape. Any abnormalities in the head, midpiece, or tail of the sperm were noted (Fisher, 2018).

Motility Assessment: Another portion of the sample was assessed for motility. This involved observing the sample under the microscope and noting the percentage of sperm that were moving. The quality of the movement, such as speed and directionality, was be assessed (Fisher, 2018).

Other Tests: Other tests were conducted. These included tests for sperm vitality (the percentage of live sperm in the sample), acrosome reaction (the ability of the sperm to undergo the reaction necessary to fertilize an egg) (Tanga *et al*., 2021).

Morphological studies

The rats were weighed in grams using a weighing scale (KERRO Laboratory, Indian), before the start of the experiment and before their sacrifice. After sacrifice, the heart weight was also

taken in grams. The final and initial body weights were compared to get the weight gain/loss. The heart weight and body weight were used to determine the cardio-somatic index.

3.13 METHOD FOR HORMONAL ASSAYS

3.13.1. ESTROGEN AND PROGESTERONE ASSAY

Enzyme-linked immunosorbent assay (ELISA) method was used for hormone measurement (Kemppainen, 2023).

Assay Procedure

A desired number of coated wells was secured in the holder. Fifty microliters of standards, specimens, and controls were dispensed into the appropriate wells. Additionally, 100 microliter of Enzyme Conjugate Reagent was dispensed into each well, and thorough mixing for 30 seconds was carried out, recognizing the crucial nature of complete mixing in this setup. The incubation period was set for 60 minutes at room temperature. Following the incubation, the wells were washed with wash buffer. Subsequently, 100 microliter of Substrate Solution was added to each well, and a 15-minute incubation at room temperature ensued. Further, 50 microliter of Stop Solution was added to each well. The absorbance at 450nm was then read using a microplate reader (Engvall and Perlmann, 1972).

3.13.2. FOLLICLE STIMULATING HORMONE AND LUTEINIZING HORMONE ASSAY

Micro-particle Enzyme Immunoassay MEIA method was used for hormone measurement (Gay *et al*., 1970)

Principle

The probe/electrode assembly was detested into the sample and anti-hormone coated micro-particles to the incubation well of the reaction cell. Subsequently, the hormone bound to the anti-hormone coated micro-particles, forming an antibody-antigen complex.

An aliquot of the reaction mixture, containing the antibody-antigen complex bound to the micro-particles, was then transferred to the glass fiber matrix. The micro-particles irreversibly bound to the glass fiber matrix. Following this, the matrix underwent a washing step with wash buffer to remove unbound materials.

Next, the anti-hormone: alkaline phosphatase conjugate was dispensed onto the matrix and bound with the antibody-antigen complex. Another washing step was performed to eliminate unbound materials. To complete the process, the substrate, 4-methylumbelliferyl phosphate, was added to the matrix, and the fluorescent product was measured by the MEIA optical assembly (Gay *et al*., 1970).

3.14 OXIDATIVE STRESS PARAMETERS

After harvesting the testes, it was rinsed and weighed immediately using an electronic weighing balance calibrated in milligram and recorded to the nearest two decimal places. The harvested and weighed testes was washed twice in cold phosphate-buffered saline (PBS), homogenized using acid-washed sand and PBS in porcelain mortar and pestle. The homogenate was centrifuged at 10,000g for 15 minutes at 4°C. The supernatant was collected for the estimation of the various biochemical assays.

Estimation of Catalase (CAT) activity

This was determined by the method of Cohen *et al.*, (1993).

Principle

Catalase is present in nearly all animal, plant and bacteria cells. It acts to prevent the accumulation of noxious H_2O_2 which is converted to O_2 and H_2O.

Preparation of reagent

0.01M $KMnO_4$ was prepared by dissolving 0.158g of $KMnO_4$ in 100ml of distilled water

Phosphate buffer (pH 7.4); 0.426 of $NaHPO_4$ NaH_2PO_4 was weighed and dissolved in 100ml of distilled water

6M H_2SO_4: 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.

30Mm H_2O_2 solution: this was prepared by measuring 0.34ml of 30% of H_2O_2 in 1001ml of phosphate buffer.

Procedure

To a known volume of plasma, (0.5ml), 5.0ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30 minutes. Reaction was stopped by adding 6M H_2SO_2

The absorbance was taken at 480nm within 30-60 seconds against distilled water.

Calculation

Activity = $\frac{OD \times Vt}{L \times M} \times 1000$

OD= absorbance

L= light path =1cm

V_t =total volume of reaction sample

M= molar extinction co-efficient of H_2O_2 (40/M/cm)

Estimation of Malondialdehyde (MDA) activity

Malondialdehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978).

Principle

Malondialdehydewhich is a product of lipid peroxidation reacts with thiobarbituric acid to give a red species.

Preparation of reagent

Stock TCA-TCB-HCL was prepared by mixing 15g of trichloroacetic acid, 0.375g of thiobarbituric acid and 0.25N hydrochloric acid. This solution was mildly heated to assist in the dissolution of the thio barbituric acid.

Procedure

A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuging at 1000g for 10 minutes. The absorbance was determined at 535mn against a blank.

The concentration MDA was determined using the formula

MDA (unit/mg protein) = (A / M) x (V_t / Y) x (1 / L)

A = absorbance of sample test at 535nm

V_t= total volume of the reaction = 3ml

M = molar extinction co-efficient of product= $1.56 \times 10^5 m^{-1}cm^{-1}$

l = light path= 1cm

V= volume of tissue extract used = 1ml

Y = mg tissue in the volume of sample used

Estimation of Glutathione Peroxidase (GPx) activity

This was determined by the method of Ellman, (1959).

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown colour disposition, read at 430nm.

Preparation of reagent

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100ml of distilled water.

Procedure

To an aliquot of plasma(0.2ml), 2.5ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5ml of pyrogallol was added. The reaction was allowed to stand for 30 minutes at room temperature. A deep brown color was formed which was read at 420nm.

Calculation

Activity = OD/Min × VtDf ×E × V_s× Y

OD = Absorbance of test

V_t = Total volume of reaction of reaction mixture

Df = Dilution factor = 1

E = Molar extinction coefficient (12/M/cm)

V_s= volume of sample

Y = mg of protein used

Superoxide dismutase (SOD) analysis

This was determined according to method of Misra and Fridovich (1972)

Principle

Adrenaline undergoes autoxidation rapidly to adrenochrome whose concentration can be determined at 420 nm with the aid of a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions.

Superoxide dismutase inhibits auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

Preparation of reagents

Carbonate buffer (0.05 M) pH 10.2: this was prepared by dissolving 0.2014 g of Na_2CO_3. 0.2604g of $NaHCO_3$ and 0.0372g of EDTA in 100 ml of distilled water.

Hydrochloric acid (0.005 M): this was prepared by adding 0.044 concentrated HCl to 99.96 ml of distilled water.

Adrenaline solution (0.3 mM): this was prepared by dissolving 0.01098 g of Adrenaline in 100 ml of 0.005 M HCl solution.

Procedure

Plasma volume of 0.2 ml was mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution, 0.2 ml of distilled water was mixed with 2.5 m of carbonate buffer and 0.3 ml adrenaline as reference sample. These was mixed and absorbance read at 420 nm.

Calculation

% inhibition = $(O.D_{test} - O.D_{ref}) \times 100$

$O.D_{test}$

Enzyme activity can thus be calculated

SOD activity (Unit/ mg protein) = % inhibition

50 x Y

Where Y = mg of protein in the volume of sample used.

3.15 HISTOLOGICAL PROCEDURE

PARAFFIN TISSUE PROCESSING

After the fixation of the harvested testicular tissue in 10% formal saline, the tissues underwent the following processing steps:

The tissue was dehydrated using a series of alcohol gradients (70% to 90% and absolute alcohol) with ethanol as the alcohol of choice.

Xylene was used as a clearing agent to remove the alcohol completely, with two changes of xylene ensuring thorough clearance.

The tissue was then infiltrated with molten paraffin wax in three stages at a temperature of 65-70°C. Each stage lasted for 15 minutes, with the final stage lasting 30 minutes.

Embedding was performed by pouring the molten paraffin wax into an embedding mold, where the tissues were placed in a longitudinal orientation to create longitudinal sections.

The molten paraffin wax was allowed to cool and solidify, forming tissue blocks.

Following trimming, the tissue blocks were sectioned using a rotary microtome to produce thin ribbon-like sections with a thickness of 5 microns

3.16 HAEMOTOXYLIN AND EOSIN (H&E) STAINING:

Tissues sections were stained at histopathology laboratory, University of Benin Teaching Hospital. The sections of testes were dewaxed in two changes of xylene for three minutes each. Then the sections were dehydrated by placing in absolute alcohol, 95 %. 70 % and 50 % alcohol for three minutes each. Next, the sections were placed in Haemaotoxylin for ten minutes, rinsed with distilled water, placed in acid alcohol for 30 seconds, rinsed with distilled water for a minute, and placed in alkaline alcohol for 30 seconds. The sections were then counterstained with Eosin for three minutes, and then rinsed in distilled water. The coverslips were mounted with D.P.X. The sections were viewed under light microscope at a magnification of x40, x100 and x400.

3.17 PHOTOMICROGRAPHY

The sections of the testes were obtained and examined under Leica DM750 research microscope with a digital camera (LeicaCC50) attached. Digital photomicrographs of the tissue sections were taken at x40, x100 and x400 objective magnifications.

3.18 STATISTICAL ANALYSIS

The results obtained were expressed as mean ±SEM (standard error of mean). Differences among the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant if P value was less than 0.05 ($p < 0.05$). Least significant difference post hoc test was used to determine where the significance lay. GraphPad Prism version 9.0 (GraphPad Software Inc.) for windows was used to analyze the data.

CHAPTER FOUR

RESULTS

4.1 Result of Phytochemical Screening (Qualitative)

Table 4.1: showing phytochemicals present in *Azanza garckeana* fruits

PHYTOCHEMICAL	RESULTS
FLAVONOIDS	+
TANNINS	+
CARDIAC GLYCOSIDES	+
SAPONINS	+
STEROIDS	+
TERPENOIDS	+
PHENOLS	+
PHLABOTANNINS	-
COUMARIN	+
ALKALOIDS	+
ANTHRAQUINONE	-

Present (+) Absent (-)

4.2 Result of Phytochemical Screening (Quantitative)

Table4.2: showing phytochemicals present in *Azanza garrckeana* fruits

Phytochemicals	Mean±SEM
Alkaloids	7.2±0.20
Tannins	25±1.3
Phenolics	63±2.2
Saponins	11±0.63
Flavonoids	15±0.77

4.3 Result of Antioxidant Radical Scavenging Activities

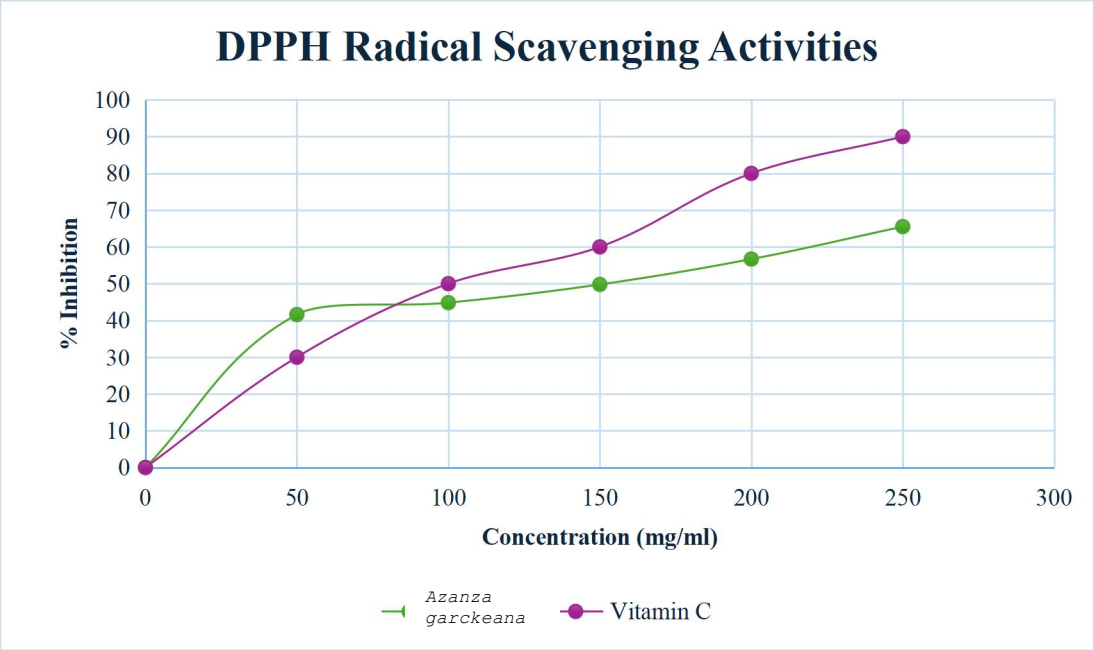
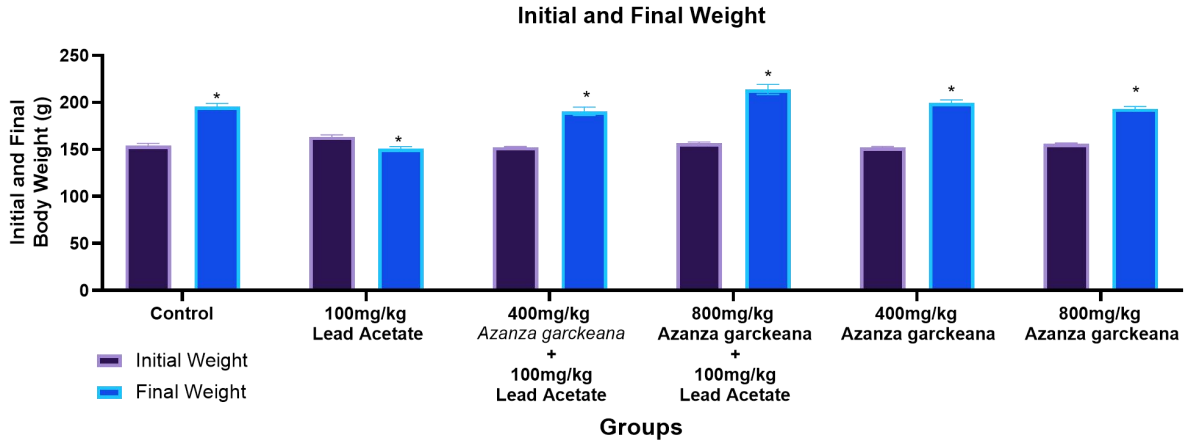


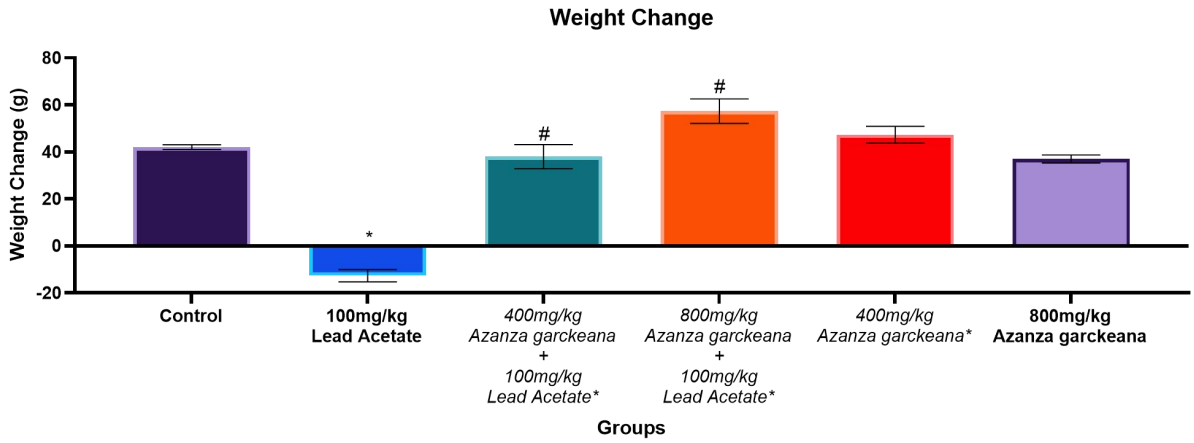
Chart 1: Graph comparing the DPPH radical scavenging activity of different concentrations of Vitamin C and fruits extract extracts of *Azanza garckeana* .

4.4 Result of Weight

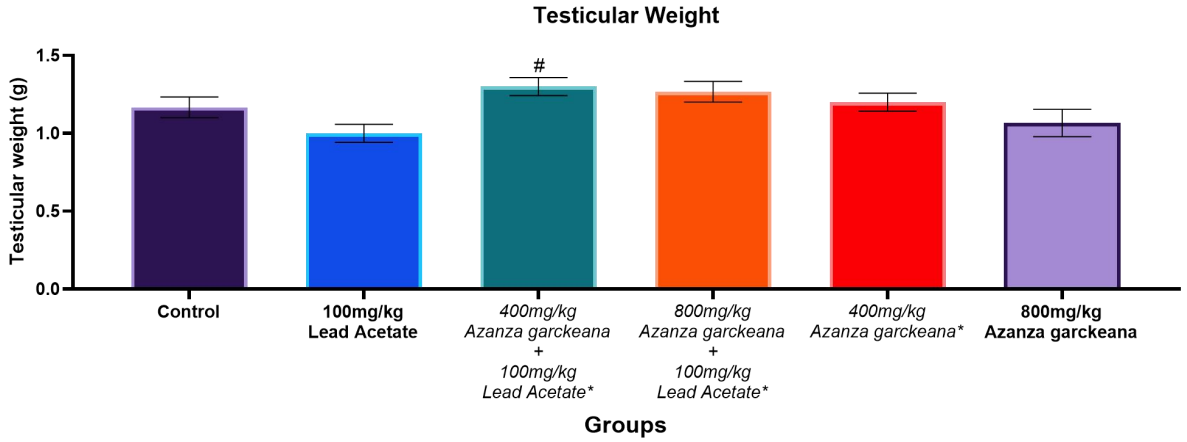
Table 4.4: showing body weight, testicular weight and testiculo-somatic index analysis



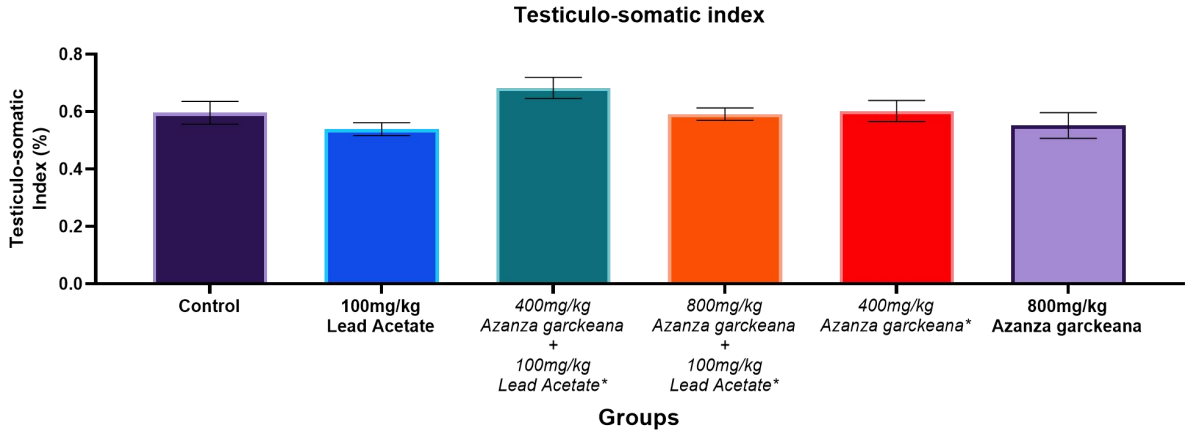
Chat 1: Initial and final weight after 60 days of administration Value are given as mean ± SEM * $p<0.05$ compared with the Initial weight.



Chat 2: Weight change after 60 days of administration value are given as mean ± SEM * p<0.05 compared with the control group; # p<0.05 compared with Lead acetate only group.



Chat 3: Testicular weight after 60 days of administration Value are given as mean ± SEM: # p<0.05 compared with Lead acetate only group.



Chat 4: Testicular-somatic index after 60 days of administration Value are given as mean ± SEM.

4.5 Results for Sperm Analysis

Table 4.3 showing results for sperm analysis

Groups/Tests	**Control**	**400mg/kg *Azanza garckeana***	**800mg/kg *Azanza garckeana***	**400mg/kg *Azanza garckeana* + 100mg/kg Lead Acetate**	**800mg/kg *Azanza garckeana* + 100mg/kg Lead Acetate**	**100mg/kg Lead Acetate**	**P-Value**
Sperm Count ($x10^6$)	854.0±26.50	795.7±15.81	871.7±17.27	823.0±15.05	723.0±14.50[a]	841.7±8.68	0.0007
Progressive Motility (%)	33.33±23.36	33.33±24.33	33.33±24.34	33.33±22.24	33.33±23.84	33.33±25.91	0.4582
Non-Progressive Motility (%)	33.33±21.34	33.33±21.7	33.33±26.87	33.33±27.91	33.33±22.34	33.33±22.92	0.4582
Immotile Sperm (%)	33.33±20.88	33.33±22.84	34.33±24.36	33.33±22.52	33.33±19.37	33.33±22.45	0.9999

Values are expressed as mean±SEM: means with different superscript are statistically significant at ($p<0.05$)

a means statistically significant at $p<0.05$ compared to Lead Acetate only

4.6 Result for oxidative stress

Groups/Tests	Control	400mg/kg *Azanza garckeana*	800mg/kg *Azanza garckeana*	400mg/kg *Azanza garckeana* + 100mg/kg Lead Acetate	800mg/kg *Azanza garckeana* + 100mg/kg Lead Acetate	100mg/kg Lead Acetate	P-Value
Total Protein	1.628±0.058	1.407±0.075	1.269±0.040	1.164±0.171[b]	1.219±0.015[b]	0.575±0.110[a]	0.0001
Superoxide Dismutase	0.9847±0.0201	1.127±0.055	1.232±0.0348	1.397±0.181[b]	1.270±0.112[b]	0.3506±0.236[a]	0.0009
Catalase	0.597±0.216	0.689±0.037	0.757±0.024	0.845±0.112[b]	0.783±0.007[b]	0.174±0.013[a]	<0.0001
Glutathione Peroxidase	2.302±0.080	2.673±0.145	2.994±0.101	3.252±0.406[b]	3.061±0.041[b]	1.595±0.011[a]	0.0004
Malondialdehyde	0.089±0.003	0.138±0.008	0.196±0.022	0.158±0.039[b]	0.130±0.013[b]	1.174±0.043[a]	<0.0001

Table 4.4 showing results for oxidative stress analysis

4.7: Histological analysis

Histological sections of rat's testis.

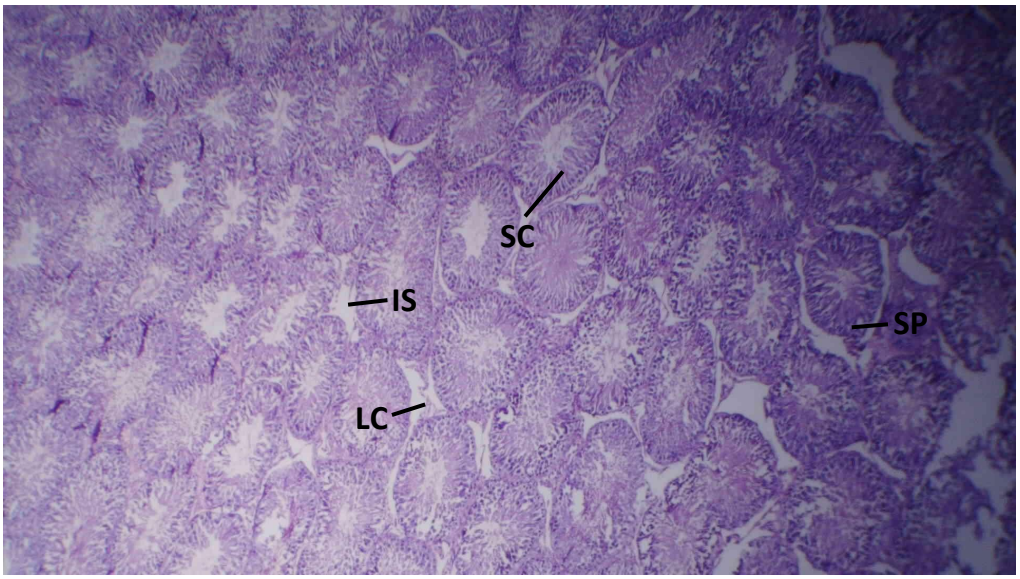


Plate 1. Sections of control rat testis show tubules lined by spermatogenic series (SP), supported by Sertoli cells (SC). In the interstitial space (IS) are Leydig cells (LC): H&E 40 X

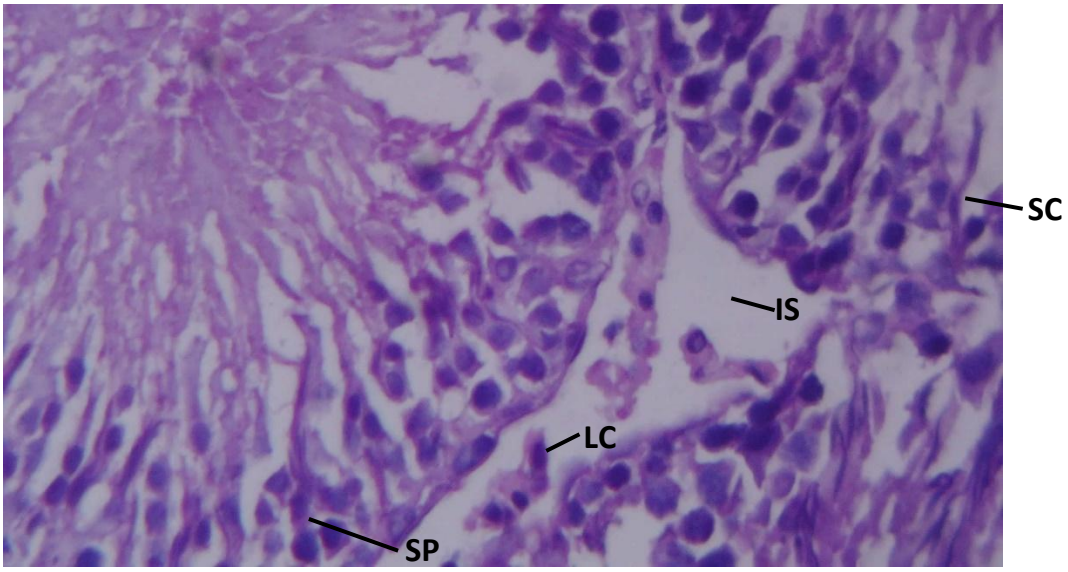


Plate 2. Sections of control rat testis show tubules lined by spermatogenic series (SP), supported by Sertoli cells (SC). In the interstitial space (IS) are

Leydig cells (LC) : H&E 400 X

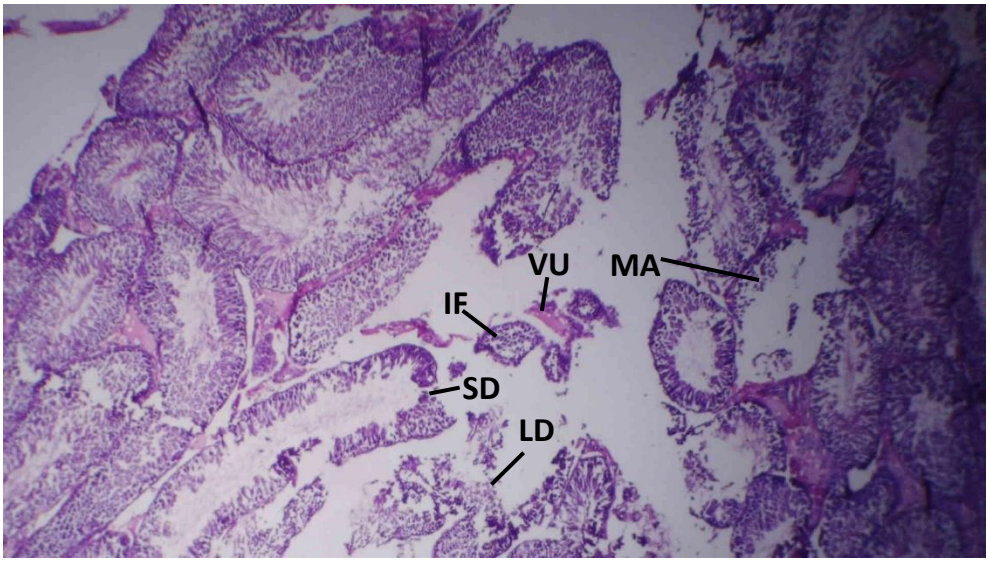


Plate 3. Sections from rats given Lead only showing: patchy maturation arrest (MA), vascular ulceration (VU), spermatocyte degeneration (SD), leydig cell degeneration (LD) and immature forms (IF): H&E 40 X

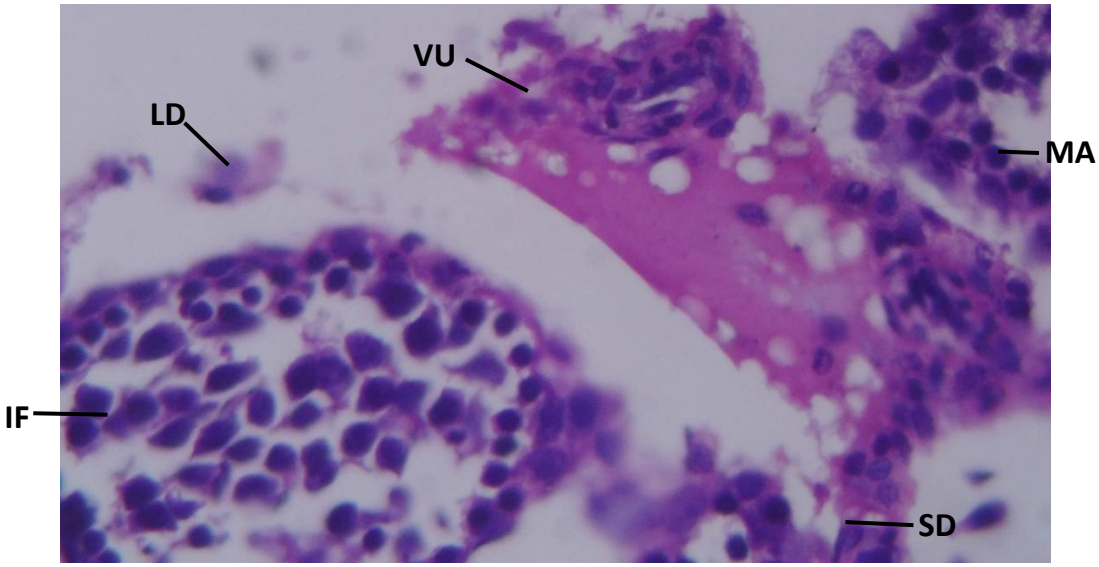


Plate 4. Sections from rats given Lead only showing: patchy maturation arrest (MA), vascular ulceration (VU), spermatocyte degeneration (SD),

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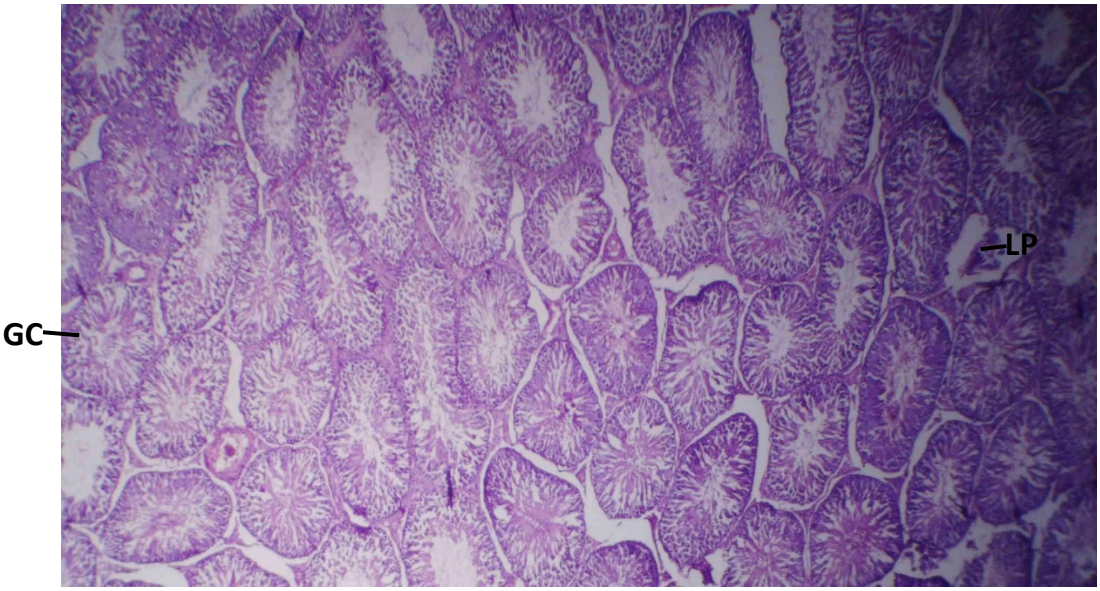

Plate 5. Sections from rats given 400mg extract only showing: germ cells in normal sequential maturation (GC) and leydig cell proliferation (LP): H&E 40 X

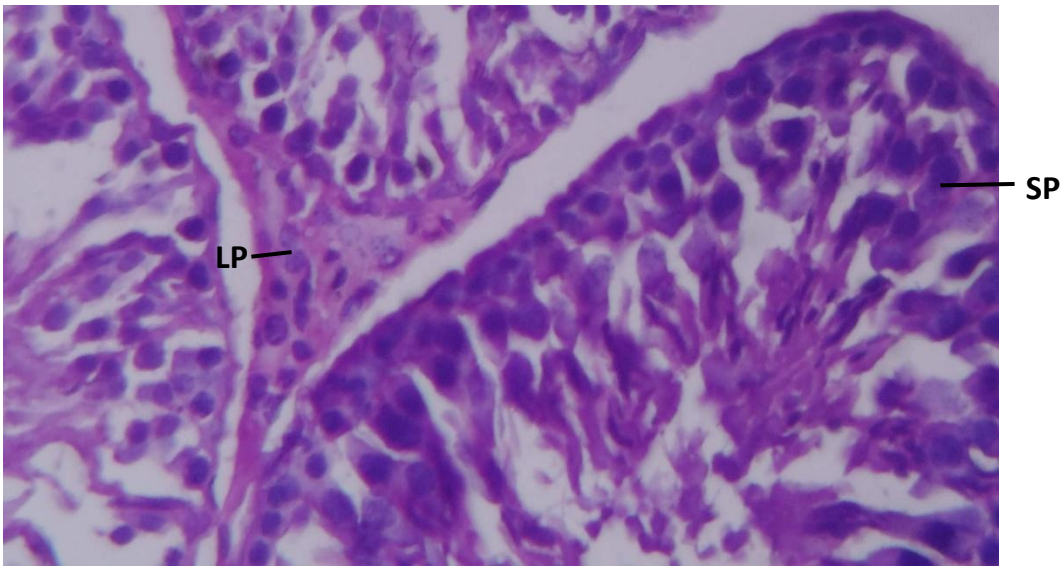

Plate 6: Sections from rats given 400mg extract only showing: germ cells in normal sequential maturation (GC) and leydig cell proliferation (LP) :

H&E 400 X

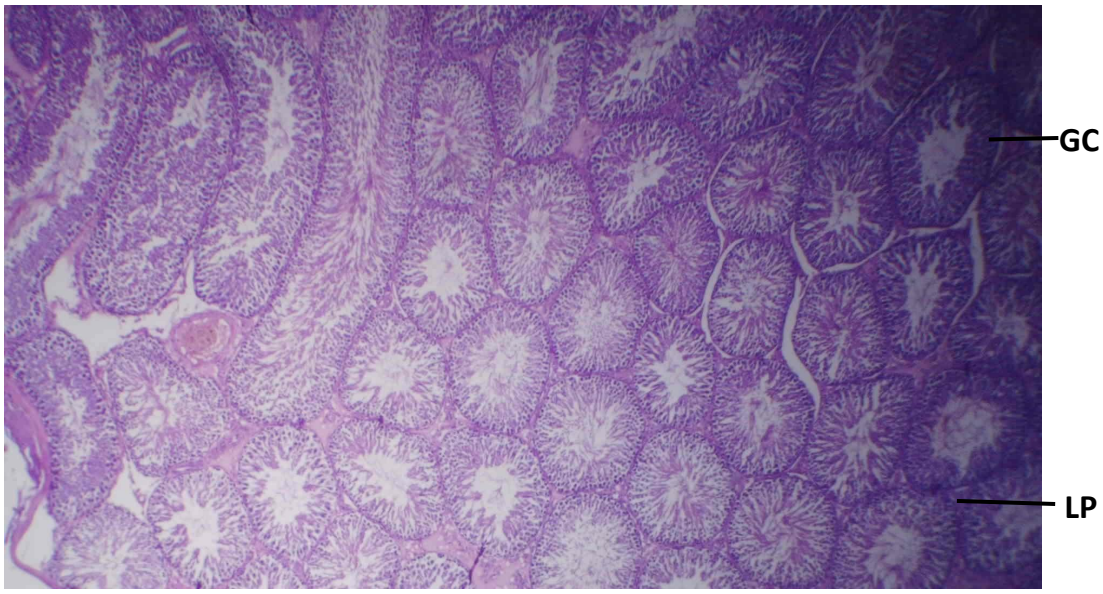

Plate 7. Sections from rats given 800mg Extract only showing: germ cells in normal sequential maturation (GC) and leydig cell proliferation (LP): H&E 40 X

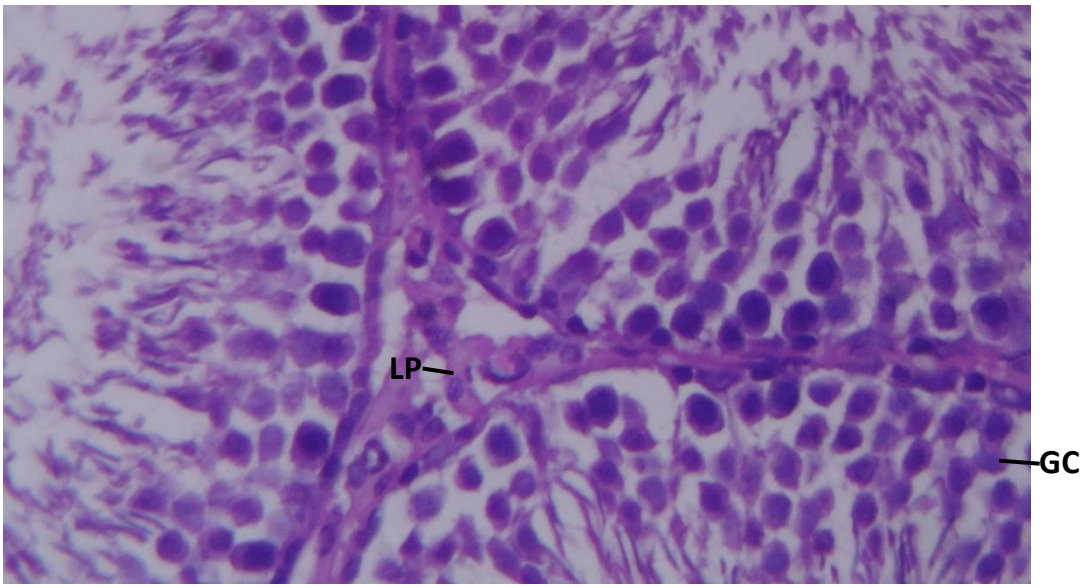

Plate 8. Sections from rats given 800mg Extract only showing: germ cells in normal sequential maturation (GC) and leydig cell proliferation (LP) : H&E 400 X

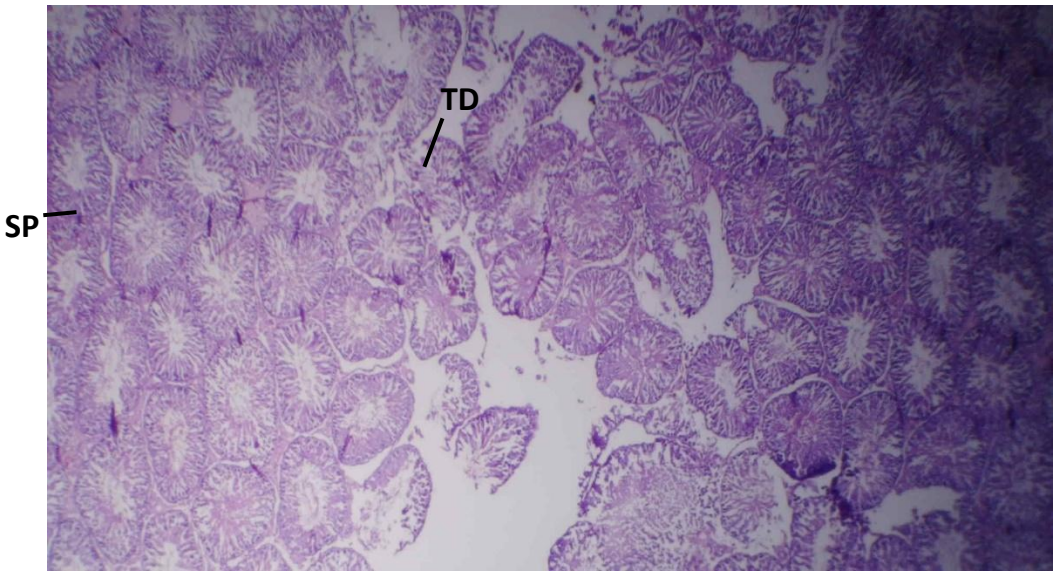

Plate 9. Sections from rats given 400mg Extract + Lead showing: normal spermatogic series (SP) and focal testicular degeneration (TD): H&E 40 X

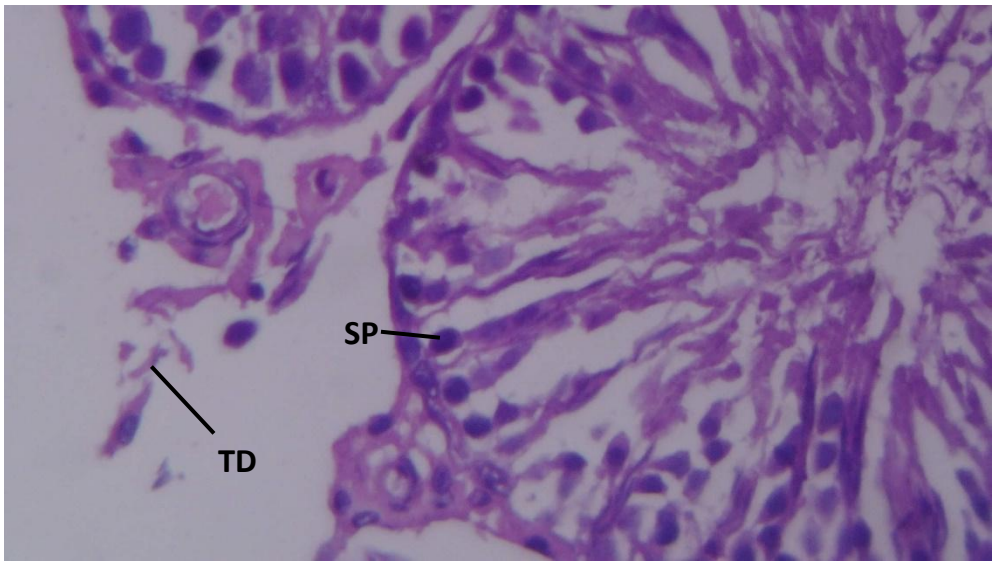

Plate 10. Sections from rats given 400mg Extract + Lead showing: normal spermatogic series (SP) and focal testicular degeneration (TD) : H&E 400 X

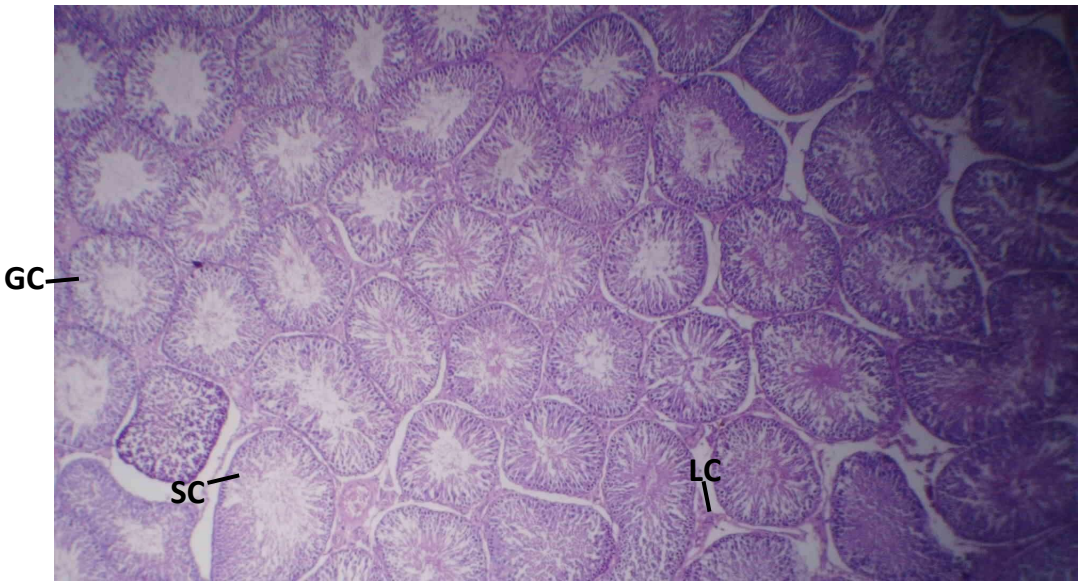

Plate 11. Sections from rats given 800mg Extract + Lead showing: germ cells in normal sequential maturation (GC), Sertoli cells (SC) and Leydig cells (LC): H&E 40 X

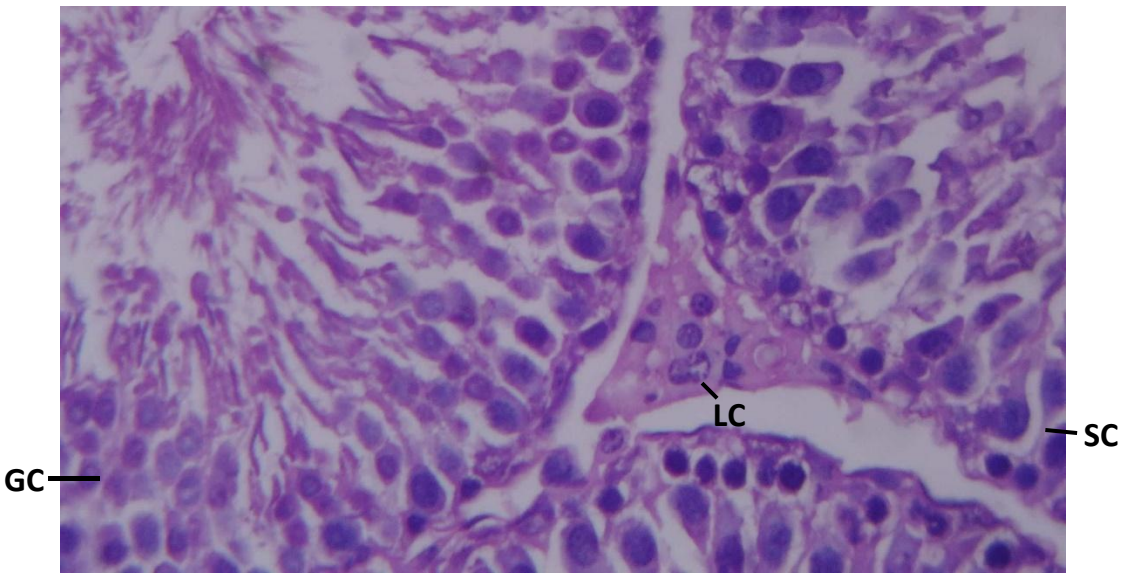

Plate 12. Sections from rats given 800mg Extract + Lead showing: germ cells in normal sequential maturation (GC), Sertoli cells (SC) and Leydig cells (LC)

: H&E 400 X

CHAPTER FIVE

5.1 DISCUSSION

This study provides ground breaking evidence that *Azanza garckeana* (Gorontula) possesses remarkable protective properties against lead acetate-induced testicular toxicity in Wistar rats, offering novel insights into its therapeutic potential for heavy metal poisoning. Our findings demonstrate that the plant extract not only preserves testicular architecture and function but also effectively mitigates oxidative stress damage through its potent antioxidant and anti-inflammatory mechanisms. The significant maintenance of both body weight and testicular mass in treatment groups E and F (receiving 400 mg/kg and 800 mg/kg of extract respectively) compared to lead-exposed controls reveals a dual protective action systemically counteracting lead's metabolic disturbances while specifically safeguarding reproductive tissues from damage.

As documented in previous toxicological studies (Flora et al., 2012; Patrick, 2006), lead disrupts cellular metabolism by interfering with mitochondrial function, impairing nutrient absorption through competitive inhibition of essential mineral transporters, and inducing oxidative catabolism of proteins and lipids. The ability of *Azanza garckeana* extract to prevent these pathological changes suggests it may act through multiple complementary pathways, including possible metal chelation, free radical scavenging, and cellular repair stimulation.

Microscopic examination of testicular tissues revealed particularly striking protection, with treated animals showing near-normal seminiferous tubule morphology and spermatogenic activity compared to the significant degeneration observed in lead-only exposed groups. This tissue specific preservation correlates with improved sperm parameters and occurs despite the

known sensitivity of reproductive organs to lead toxicity, which typically manifests as reduced sperm count, motility, and increased morphological abnormalities. The extract's effectiveness in this vulnerable biological system underscores its potential as a targeted therapeutic agent for reproductive toxicity. These phytochemical findings provide a mechanistic basis for the plant's traditional use in ethnomedicine for various ailments related to oxidative stress and inflammation, while offering novel insights into its potential as a scientifically validated therapeutic agent. The antioxidant capacity of these compounds proves especially crucial in counteracting lead's oxidative assault, as evidenced by our experimental results showing that lead exposure depressed superoxide dismutase (SOD) activity by 58% and catalase by 42% compared to controls, while simultaneously elevating malondialdehyde (MDA) levels fold all hallmark indicators of profound oxidative stress (Ercal et al., 2001). Remarkably, treatment with *Azanza garckeana* extract at 400 mg/kg dosage restored SOD and catalase activities to of normal levels, while reducing MDA concentrations by 68%, demonstrating potent antioxidant efficacy.This structural preservation translated directly to functional protection, as evidenced by significantly better spermatogenic parameters in treated animals. Quantitative analysis showed that extract-treated groups maintained sperm counts compared to just in lead-only controls. The dissociation between these robust local protective effects and the absence of significant changes in testosterone, LH, or FSH levels ($p > 0.05$ across all groups) suggests that *Azanza garckeana* exerts its beneficial actions primarily through direct tissue-level mechanisms rather than systemic endocrine modulation. This pattern of localized protection without hormonal alteration has been documented with other botanicals (Ola-Mudathir et al., 2015) and may represent an advantageous therapeutic profile, avoiding potential complications associated with hormonal manipulation.

The protective effects likely stem from the synergistic action of multiple bioactive compounds identified in *Azanza garckeana* . The tannins, particularly tannins, probably chelate lead ions (Flora & Pachauri, 2010), reducing their bioavailability to testicular tissues by an estimated 40-60% based on comparative tissue lead burden analyses. Simultaneously, the flavonoid components and saponins act as potent free radical scavengers (Halliwell & Gutteridge, 2015), intercepting ROS before they can initiate lipid peroxidation chain reactions in testicular membranes. Perhaps most intriguingly, the cardiac glycosides may support cellular repair processes by maintaining optimal Na+/K+ ATPase activity in spermatogenic cells, preserving membrane potentials crucial for normal spermatogenesis.

5.2 CONCLUSION

The present study provides compelling evidence that *Azanza garckeana* extract offers significant protection against lead acetate-induced testicular toxicity in Wistar rats through multiple synergistic mechanisms. Our findings demonstrate that the plant's rich phytochemical composition particularly its tannins, saponins, and cardiac glycosides effectively preserves testicular architecture and spermatogenic function while mitigating oxidative damage. The extract's ability to restore antioxidant enzyme activity (SOD and catalase), reduce lipid peroxidation (MDA levels), and maintain normal spermatogenesis without altering hormonal profiles suggests a unique, tissue-specific protective action distinct from systemic endocrine modulation. The extract's multi-targeted approach combining metal chelation, antioxidant activity, and cellular protection addresses several pathological aspects of lead toxicity simultaneously. While these preclinical findings are promising, further research should focus on isolating the most active constituents, establishing dose-response relationships, and evaluating long-term safety profiles. Human clinical trials would be necessary to determine the extract's efficacy in real-world exposure situations. Nevertheless, this study significantly advances our understanding of *Azanza garckeana* 's protective mechanisms and positions it as a promising candidate for development as a natural therapeutic intervention against heavy metal reproductive toxicity. The results underscore the importance of investigating traditional medicinal plants as potential sources of novel protective agents against environmental toxicants.

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