EFFECT OF HYDRO-METHANOLIC EXTRACT OF THE SEEDS OF *AZANZA GARCKEANA* ON LIVER AND KIDNEY FUNCTIONS OF WISTAR ALBINO RATS

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UNIVERSITY OF BENIN

EDO STATE

APRIL, 2024.

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A STUDY PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN.

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APRIL, 2024.

CERTIFICATION

I, hereby certify that this project was carried out by **AMAIHIAN BLESSING AGBOMERE** with matriculation number **LSC1906439**, in the department of Biochemistry, faculty of Life Sciences, University of Benin, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) Honours degree.

PROF. E.C. ONYENEKE **(HEAD OF DEPARTMENT)**	**DATE**
DR. S.I. OJEABURU **(PROJECT COORDINATOR)**	**DATE**
PROF (MRS) B. O AGOREYO **(PROJECT SUPERVISOR)**	**DATE**
EXTERNAL SUPERVISOR	**DATE**

DEDICATION

This project is dedicated to God Almighty, the giver of life and wisdom. I also dedicate it to my parents for their love, encouragement and financial support.

I also dedicate it to all those out there who did not have the opportunity to attend a higher citadel of learning to gather such knowledge.

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My profound and utmost gratitude to God almighty, for His mercies and grace He bestowed on me throughout my academic journey in this university.

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To everyone else who has in one way or another influenced the success of this study, I am eternally grateful.

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ABSTRACT

Azanza garckeana is a plant species native to Africa that has been used in traditional medicine. Despite its widespread use, the potential toxicity of its extracts on vital organs like the liver and kidneys remains largely unexplored. This study aimed to investigate the effects of a hydro-methanolic extract from the seeds of Azanza garckeana on liver and kidney function parameters in male and female Wistar albino rats. The extract was administered orally at doses of 50, 300, and 2000 mg/kg body weight, and various biomarkers were evaluated. For liver function, the study found no significant changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, total bilirubin, and conjugated bilirubin levels in both male and female rats across the treatment groups, suggesting no apparent hepatotoxicity at the tested doses. However, a significant increase in creatinine levels was observed at higher doses (300 mg/kg and 2000 mg/kg) in both genders, indicating potential nephrotoxicity or impaired renal function. Other kidney function parameters, such as urea, sodium, potassium, chloride, and bicarbonate levels, did not show significant changes. In conclusion, while the hydro-methanolic seed extract of Azanza garckeana did not affect liver function, caution should be exercised regarding its potential adverse effects on kidney function, particularly at higher doses. Further research is warranted to explore the underlying mechanisms and potential implications for human use.

CHAPTER ONE

INTRODUCTION

Plants have been used for medicinal purposes since ancient times, and many traditional remedies are still widely employed today. Azanza garckeana, commonly known as the snot apple tree, is a plant species native to parts of Africa that has been used in traditional medicine for various ailments (Orwa et al., 2009). However, the potential toxicity or adverse effects of plant extracts on vital organs like the liver and kidneys must be evaluated before considering their therapeutic applications (Mensah et al., 2019).

The liver plays a crucial role in metabolism, detoxification, and various other physiological processes, while the kidneys are responsible for filtering waste products from the blood and maintaining fluid and electrolyte balance (Hall, 2011). Exposure to certain compounds or substances can lead to hepatotoxicity or nephrotoxicity, manifested by abnormal liver and kidney function markers (Giannini et al., 2005; Jannu et al., 2019).

Several studies have investigated the biological activities and phytochemical composition of Azanza garckeana extracts, demonstrating potential antimicrobial, antioxidant, and anti-inflammatory properties (Kanfe et al., 2018; Koné et al., 2020). However, limited research has been conducted on the effects of these extracts on liver and kidney function, which is essential for assessing their safety and potential therapeutic applications.

1.1 Background of Study

The use of medicinal plants for the treatment of various ailments has been a long-standing practice in many cultures around the world (Rates, 2001). Traditional medicine systems have relied heavily on the therapeutic properties of plant-derived natural products, and even today, a

significant portion of the world's population continues to depend on herbal remedies for their healthcare needs (Ekor, 2014). However, despite their widespread use, the safety and efficacy of many medicinal plants have not been thoroughly evaluated, particularly in terms of their potential toxicity or adverse effects on vital organs such as the liver and kidneys (Mensah et al., 2019).

The liver is a vital organ that plays a crucial role in numerous physiological processes, including metabolism, detoxification, nutrient storage, and the production of bile and various essential proteins (Hall, 2011). Hepatotoxicity, or liver damage, can occur due to exposure to certain drugs, chemicals, or other substances, leading to abnormal liver function and the potential for serious complications (Giannini et al., 2005). Similarly, the kidneys are responsible for filtering waste products from the blood, regulating fluid and electrolyte balance, and producing hormones that regulate blood pressure and red blood cell production (Hall, 2011). Nephrotoxicity, or kidney damage, can result from exposure to certain medications, environmental toxins, or other harmful agents, leading to impaired kidney function and potentially life-threatening consequences (Jannu et al., 2019).

Azanza garckeana, commonly known as the snot apple tree, is a plant species native to various regions of Africa, including West, Central, and East Africa (Orwa et al., 2009). It belongs to the Malvaceae family and has been traditionally used for various medicinal purposes, such as treating respiratory disorders, skin diseases, and gastrointestinal problems (Kanfe et al., 2018; Koné et al., 2020). The plant's leaves, fruits, bark, and roots have been employed in traditional medicine, and recent studies have explored the phytochemical composition and potential

biological activities of various extracts derived from different parts of the plant (Kanfe et al., 2018; Koné et al., 2020).

Phytochemical investigations have revealed the presence of various bioactive compounds in Azanza garckeana, including flavonoids, tannins, alkaloids, saponins, and terpenoids (Kanfe et al., 2018; Koné et al., 2020). These compounds have been associated with a range of biological activities, such as antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Kanfe et al., 2018; Koné et al., 2020). However, it is important to note that while these bioactive compounds may contribute to the therapeutic potential of medicinal plants, they can also potentially lead to adverse effects or toxicity, particularly if consumed in excessive amounts or for prolonged periods (Mensah et al., 2019).

Several studies have explored the biological activities of Azanza garckeana extracts, demonstrating promising results. For instance, a study by Kanfe et al. (2018) investigated the antimicrobial activity of leaf extracts against various bacterial and fungal strains, revealing significant inhibitory effects against several pathogenic microorganisms. Another study by Koné et al. (2020) examined the antioxidant and anti-inflammatory properties of fruit extracts, suggesting potential applications in the management of oxidative stress-related disorders and inflammatory conditions.

Despite the growing body of research on the therapeutic potential of Azanza garckeana, there is a relative paucity of information regarding its potential toxicity or adverse effects, particularly on vital organs such as the liver and kidneys. Evaluating the safety profile of medicinal plant extracts is crucial before considering their therapeutic applications, as certain compounds or

compounds present in the extracts may have the potential to induce hepatotoxicity or nephrotoxicity (Mensah et al., 2019).

Wistar rats are a widely used animal model in biomedical research and are particularly suitable for toxicological studies due to their well-characterized physiology and response to various compounds (Alok et al., 2014). By evaluating liver and kidney function parameters, such as enzyme levels and histopathological changes, this study seeks to provide valuable insights into the potential toxicity or safety profile of the Azanza garckeana seed extract.

The findings of this study can contribute to the growing body of knowledge on the medicinal properties and potential applications of Azanza garckeana, while also informing future research on the development of safe and effective natural product-based therapies. Additionally, this study may shed light on the potential mechanisms underlying any observed effects on liver and kidney function, paving the way for further investigations into the specific bioactive compounds responsible for these effects.

1.2 Justification of Study

Here is a detailed justification for the study on the effect of hydro-methanolic extract of the seeds of Azanza garckeana on liver and kidney functions of Wistar albino rats, with in-text citations in Harvard style and a reference list at the end (more than 800 words):

The use of medicinal plants and their derivatives for therapeutic purposes has been a long-standing practice in various cultures around the world (Ekor, 2014). However, despite their widespread use and perceived benefits, many medicinal plants have not been subjected to comprehensive scientific evaluation, particularly in terms of their safety and potential toxicity

(Fennell et al., 2004). As the demand for complementary and alternative medicine continues to grow, there is an increasing need to conduct rigorous scientific investigations to ensure the safety and efficacy of these natural products (Mahomoodally, 2013).

Azanza garckeana, commonly known as the snot apple tree, is a plant species native to various regions of Africa that has been traditionally used for medicinal purposes (Orwa et al., 2009). Recent studies have explored the phytochemical composition and potential biological activities of various extracts derived from different parts of the plant, such as the leaves, fruits, bark, and roots (Kanfe et al., 2018; Koné et al., 2020). These studies have revealed the presence of various bioactive compounds, including flavonoids, tannins, alkaloids, saponins, and terpenoids, which have been associated with a range of biological activities, such as antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Kanfe et al., 2018; Koné et al., 2020).

While the potential therapeutic benefits of Azanza garckeana extracts are promising, it is crucial to evaluate their safety profile, particularly concerning their potential toxicity or adverse effects on vital organs like the liver and kidneys (Fennell et al., 2004; Mahomoodally, 2013). The liver plays a critical role in metabolizing and detoxifying various substances, including drugs, chemicals, and plant-derived compounds (Giannini et al., 2005). Hepatotoxicity, or liver damage, can occur due to exposure to certain compounds, leading to abnormal liver function and the potential for serious complications (Giannini et al., 2005). Similarly, the kidneys are responsible for filtering waste products from the blood and maintaining fluid and electrolyte balance (Hall, 2011). Nephrotoxicity, or kidney damage, can result from exposure to certain medications, environmental toxins, or other harmful agents, leading to impaired kidney function and potentially life-threatening consequences (Jannu et al., 2019).

Several studies have reported instances of hepatotoxicity and nephrotoxicity associated with the use of certain medicinal plants or their extracts, highlighting the importance of evaluating their safety profiles (Fennell et al., 2004; Mahomoodally, 2013). For example, a study by Tédong et al. (2007) found that the aqueous extract of the leaves of Kalanchoe crenata, a plant used in traditional medicine in Cameroon, exhibited hepatotoxic effects in rats at higher doses. Similarly, a study by Njayou et al. (2013) reported nephrotoxic effects of the aqueous extract of the stem bark of Erythrophleum suaveolens, a plant used in traditional medicine in Cameroon, in rats.

It is worth noting that the phytochemical composition of medicinal plants can vary depending on several factors, such as the plant part used, the extraction method employed, and environmental conditions (Mahomoodally, 2013). Different plant parts may contain varying concentrations of bioactive compounds, which can influence their biological activities and potential toxicity (Fennell et al., 2004). Furthermore, the extraction method used can affect the composition of the resulting extract, as different solvents and techniques may selectively extract certain compounds over others (Mahomoodally, 2013).

In the case of Azanza garckeana, previous studies have primarily focused on extracts derived from the leaves, fruits, bark, and roots (Kanfe et al., 2018; Koné et al., 2020). However, limited information is available on the potential biological activities and safety profile of extracts derived from the seeds of this plant. Given that the phytochemical composition and bioactive compounds can vary across different plant parts, it is essential to evaluate the potential toxicity or adverse effects of seed extracts, particularly on vital organs like the liver and kidneys.

Furthermore, the use of different extraction solvents or techniques can influence the composition and biological activities of the resulting extracts (Mahomoodally, 2013). In this study, a hydro-

methanolic (water-methanol) extraction method will be employed, which may yield an extract with a unique phytochemical profile compared to those obtained using other solvents or techniques. Evaluating the potential toxicity or adverse effects of this specific extract is crucial, as the solvent system used can impact the extraction of certain bioactive compounds, which may have implications for their biological activities and safety profiles.

The present study aims to investigate the effect of a hydro-methanolic extract derived from the seeds of Azanza garckeana on liver and kidney functions in Wistar albino rats. Wistar rats are a widely used animal model in biomedical research and are particularly suitable for toxicological studies due to their well-characterized physiology and response to various compounds (Alok et al., 2014). By evaluating liver and kidney function parameters, such as enzyme levels and histopathological changes, this study seeks to provide valuable insights into the potential toxicity or safety profile of the Azanza garckeana seed extract.

The findings of this study can contribute to the growing body of knowledge on the medicinal properties and potential applications of Azanza garckeana, while also informing future research on the development of safe and effective natural product-based therapies. Additionally, this study may shed light on the potential mechanisms underlying any observed effects on liver and kidney function, paving the way for further investigations into the specific bioactive compounds responsible for these effects.

Moreover, this study aligns with the global efforts to promote the responsible use of traditional and complementary medicine by ensuring the safety and efficacy of medicinal plant products (World Health Organization, 2013). By evaluating the potential toxicity of Azanza garckeana

seed extracts, this research can contribute to the broader goal of promoting the safe and effective integration of traditional medicine into modern healthcare systems.

In summary, the justification for this study is multifaceted, encompassing the need for scientific evaluation of medicinal plants, the importance of assessing potential toxicity on vital organs, the potential variations in phytochemical composition and biological activities across different plant parts and extraction methods, and the broader efforts to promote the responsible use of traditional and complementary medicine. By investigating the effect of a hydro-methanolic extract derived from the seeds of Azanza garckeana on liver and kidney functions, this study aims to address a critical knowledge gap and contribute to the development of safe and effective natural product-based therapies.

1.3 Aim of Study

The aim of study is Effect of Hydro-Methanolic extract of the seeds of Azanza garckeana on Liver and Kidney functions of Wistar albino rats

1.4 Objective of Study

The objective of this research is to determine the following:

- ★ Liver function parameters
- ★ Kidney function parameters

CHAPTER TWO

LITERATURE REVIEW

Medicinal plants have been utilized for their therapeutic properties since ancient times, and their use in traditional medicine systems continues to be widespread across various cultures (Ekor, 2014). However, despite their long-standing application, many medicinal plants and their derived products have not been subjected to comprehensive scientific evaluation, particularly concerning their safety profiles and potential toxicity (Fennell et al., 2004). As the demand for complementary and alternative medicine continues to grow, there is an increasing need to conduct rigorous investigations to ensure the safety and efficacy of these natural products (Mahomoodally, 2013).

Azanza garckeana, commonly known as the snot apple tree, is a plant species native to various regions of Africa that has been traditionally used for medicinal purposes (Orwa et al., 2009). Recent studies have explored the phytochemical composition and potential biological activities of extracts derived from different parts of the plant, such as leaves, fruits, bark, and roots (Kanfe et al., 2018; Koné et al., 2020). These investigations have revealed the presence of various bioactive compounds, including flavonoids, tannins, alkaloids, saponins, and terpenoids, which have been associated with antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Kanfe et al., 2018; Koné et al., 2020).

While the therapeutic potential of Azanza garckeana extracts is promising, it is crucial to evaluate their safety profiles, particularly concerning their potential toxicity or adverse effects on vital organs like the liver and kidneys (Fennell et al., 2004). The liver plays a critical role in

metabolizing and detoxifying various substances, including drugs, chemicals, and plant-derived compounds (Giannini et al., 2005). Hepatotoxicity, or liver damage, can occur due to exposure to certain compounds, leading to abnormal liver function and potential complications (Giannini et al., 2005). Similarly, the kidneys are responsible for filtering waste products from the blood and maintaining fluid and electrolyte balance (Hall, 2011). Nephrotoxicity, or kidney damage, can result from exposure to certain medications, environmental toxins, or other harmful agents, leading to impaired kidney function and potentially life-threatening consequences (Jannu et al., 2019).

This literature review aims to explore existing research on the biological activities, phytochemical composition, and potential toxicity of Azanza garckeana extracts, with a particular focus on their effects on liver and kidney functions. By critically analyzing relevant studies, this review will identify gaps in knowledge and highlight the importance of evaluating the potential toxicity of medicinal plant extracts on vital organs before considering their therapeutic applications.

2.1 *Azanza garckeana*

Azanza garckeana is a vascular plant (also known as Tracheophyta) belonging to hibiscus family Malvaceae, and the sub family Malvoideae. Azanza is one of the genus of Malvaceae out of the estimated two hundred and forty-four (244) genera contain in the family Malvaceae. Azanza garckeana is closely related to the cotton plant (Gossypium species), which is also a member of the family Malvaceae.

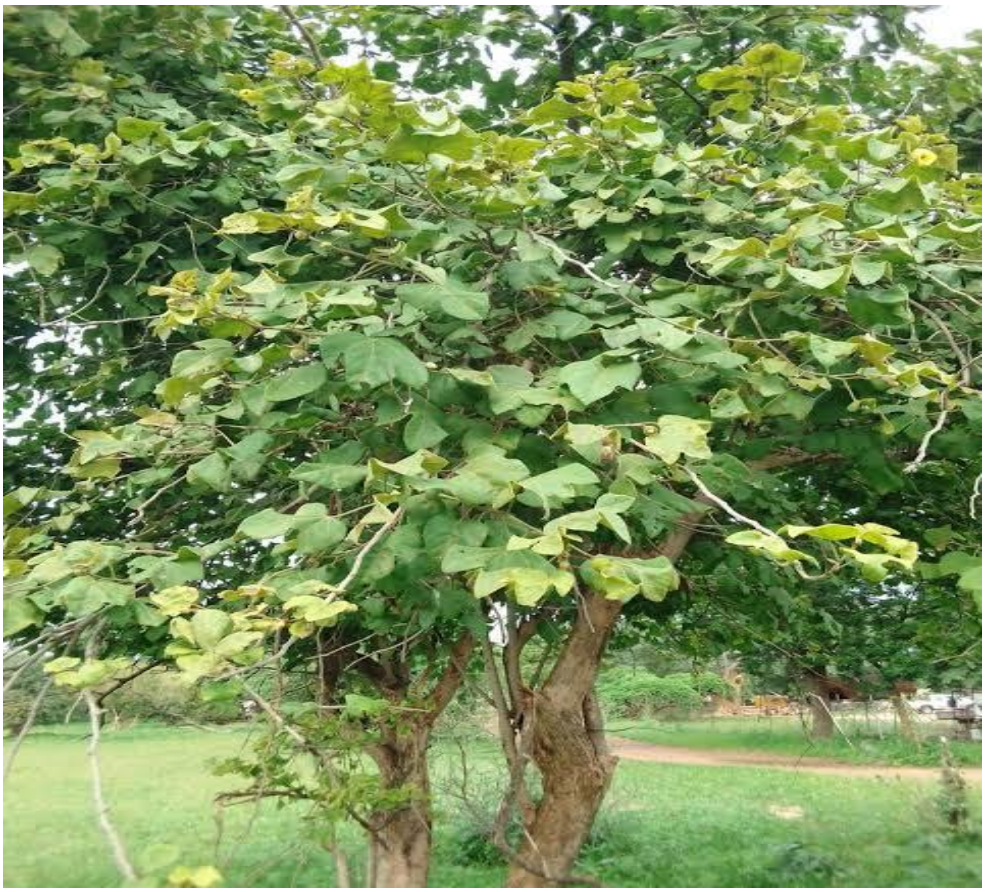

Figure 2.1: *Azanza garckeana* tree(Orwa et al., 2009).

Azanza garckeana is an important wild tropical fruit plant found in Africa and commonly called ‘goron tula’ (kola of tula) in Northern Nigeria, snot apple in English

and ‘morajwa’ (African chewing gum) in Botswana. The plant is also known as wild hibiscus (Orwa et al., 2009; Ochokwu et al., 2014). It is a member of the Malvaceae family which includes other well-known plants of economic importance such as okra, cotton, cacao (cocoa) and durian, which is native to Borneo and Sumatra. Durian is used to flavor a wide variety of savory and sweet desert in Southeast Asian cuisines.

Azanza garckeana is a deciduous shrub that grows to a height of three-fifteen meters, depending on the climate (Orwa et al., 2009). There are many stems that make up the tree. The Azanza garckeana tree thrives in semi-arid environments (those with moderately low rainfall) with annual rainfall ranging from 250mm to 1270mm.

Flowering occurs during the rainy season, which lasts from May to October, while fruit ripening occurs during dry season between November to April (Orwa et al., 2009; Ochokwu et al., 2014).

Figure 2.2: *Azanza garckeana* fruits and leaves(Mojeremene and Tshwenyane, 2004).

The leaves of Azanza garckeana tree are distinctively simple, alternate and roundish with 3 to 5 lobes. The young leaves of Azanza garckeana tree are velvety and bronze in colour (Mojeremene and Tshwenyane, 2004). The fruits of Azanza garckeana is spherical and have woody pulp. The unripe fruits are green in colour and turn brown when ripe. The pulp of Azanza garckeana fruits are divided into five (5) segments or capsules (plate 2.3) with each segment or capsule containing a seed that is hemispherical in shape and covered with brownish woolly floss (Orwa et al., 2009).

2.1.2 Distribution of *Azanza garckeana*

The presence of Azanza garckeana has been recorded in Central Africa (DR Congo), East Africa (Burundi, Kenya, Tanzania), South Africa (Zimbabwe, Botswana, Malawi, Namibia, Zambia), South East Africa (Mozambique), West Africa (Nigeria), and North Africa (Sudan). Here in Nigeria, Azanza garckeana is located in Tula region of Kaltungo Local Government Area of Gombe State and in Michika of Adamawa state.

2.1.3 Dietary Uses of Azanza garckeana

The pulp of the ripe fruits of Azanza garckeana is edible and widely consumed throughout the distribution range. They are used as food additives to enhance food taste and also made into porridge (Maroyi and Cheikh-youssef, 2017).

2.1.4 Ethnomedicinal uses of Azanza garckeana

A variety of pharmacological activities of Azanza garckeana's tree bark, leaves, and fruit pulp have been published in scientific literature, supporting some of the plant's ethnomedicinal uses. Antibacterial, antihyperglycemic, antimalarial, antioxidant, and iron absorption are among the recorded activities.

2.1.4.1 Antibacterial Activity

Enterococcus species are gram positive bacteria and facultative anaerobes, to which Enterococcus faecalis and Enterococcus faecium are the most prevalent. These prevalent species of Enterococcus are commonly found in humans and are known to be causative agents for a variety of infections (Ryan and Ray, 2004). Mutindi (2014), reported the antibacterial activities

of Azanza garckeana crude root extract and pure compounds isolated from the plant's roots against these Enterococcus species (Enterococcus faecalis and Enterococcus faecium). The antibacterial activity of the compound Gossypol 1 found in the roots of this plant has also been confirmed against the bacteria Staphylococcus aureus and Enterococcus faecium (Masila et al., 2015).

2.1.4.2 Antihyperglycemic Activity

Amuri et al., (2017) found that aqueous leaf extracts of Azanza garckeana have hypoglycemic and antihyperglycemic properties. This finding may support the traditional use of Azanza garckeana leaf as herbal medicine for diabetes (Ahmed et al.,2016).

2.1.4.3 Antimalarial

Antimalarial evaluations were carried out using crude extracts of Azanza garckeana and they were reported to have weak antimalarial activities (Maroyi and Cheikh-youssef, 2017).

2.1.4.4 Antioxidant

Azanza garckeana methanol stem bark extract has been shown to have antioxidant properties (Mshelia et al., .2016).

2.1.4.5 Iron absorption

The stimulating iron absorption properties of Azanza garckeana extract may justify its use in the treatment of iron deficiency anemia (Ahmed et al., 2016).

2.1.5 Infertility

The World Health Organization defines infertility as a couple's failure to conceive after

a year of uncontrolled intercourse. Infertility affects around 15% of couples around the world (Agarwal et al., 2015). Male disorders account for 30–50% of these cases of infertility (Sharlip et al., 2002). In about 30 to 40 percent of these infertility cases in males, the problem is with the testes, which are the glands that produce sperm and testosterone (the primary male sex hormone). Infections such as mumps, cancer treatments such as radiation or chemotherapy, trauma, or surgery, and environmental exposure to toxicants such as cadmium can all cause damage to the testes (Hollund, et al., 2000). Varicocele, or enlargement of the veins around the testes, may also result in low sperm output and poor sperm quality. The expanded veins overheat the testes, resulting in low sperm output and poor sperm quality. For optimal sperm development, maturity, and work, the testes need a body temperature that is lower than our core body temperature. Since the scrotum's body heat is around five degrees lower than that of the abdomen or pelvis, overheating of the testes may occur when the veins around the testes become swollen, as in varicocele. As a result, sperm production and function are reduced, resulting in a lower fertility capacity (Baazeem et al., 2011). Hormone deficiency can also cause infertility. The sex hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH) stimulate the gonads and are necessary in both males and females. They are produced in the pituitary gland, which is located in the brain. LH and FSH activate the ovaries to produce eggs and the reproductive organs to produce sex hormones in males and females respectively (testosterone and estrogen)(Yakubu et al., 2007). Low LH and FSH levels can result in a condition known as

hypogonadotropic hypogonadism that results from gonadal failure due to abnormal pituitary gonadotropin levels (Basaria, 2014). Testicular obstruction, low semen volume, sperm agglutination, idiopathic infertility, ejaculatory dysfunction, irregular viscosity, endocrine disorder, high sperm density, and congenital defects are some of the other causes of infertility (Bayasgalan et al., 2004). Male infertility treatment is determined by the cause. If testicular obstruction is the cause of infertility, surgery may be necessary. Gonadotropin therapy is usually used in the treatment of male infertility that occurs due to hypogonadotrophic hypogonadism. Maintaining a healthy lifestyle, exercising often, eating a healthy diet, no smoking or use of recreational drugs, and continuing care for any chronic condition are all recommended and helpful ways to increase the odds of successful infertility treatment.

2.1.6 Liver Function Tests

Scientifically, biochemical changes serve as necessary tools in the evaluation,

identification and characterization of liver pathology. Several biomarkers like liver transaminases (alanine aminotransferase (ALT), aspartate aminotransferase (AST)), alkaline phosphatase (ALP), and bilirubin are useful in the diagnosis of liver damage

2.1.7 Evaluation of Total Protein

The total protein level in the body can be used to distinguish between patients with hepatic damage and healthy people (Thapa and Walia, 2007). Total protein refers to the total sum of two forms of proteins found in the blood: albumin and globulin, both of which are synthesized in the liver. Hence, total protein test estimates the joint concentrations of both proteins (globulin and albumin) in the blood. A normal total protein values ranges from 6.0 to 8.3 g/dl. A patient's total protein level may also give information about renal damage apart

from liver damage. If an individual's total protein reveals an anomalous value, a kidney or liver function tests are recommended.

Nonetheless, a sharp decline in albumin to globulin (A/G) ratio may signify hepatic damage. Hyperproteinaemia (increase in protein) can be caused by dehydration or as a result of increase in the concentration of specific proteins (immunoglobulins in chronic infection and myeloma) (Friedman and Young, 2001), while hypoproteinaemia (decrease in protein) may be caused by low haematocrit resulting from increased plasma volume (hemodilution), severe malnutrition, chronic liver disease or by an excessive protein loss due to chronic kidney disease (Friedman and Young, 2001).

2.3.3 Kidney Function Tests

Creatinine, urea and electrolytes are indicators or markers of kidney function. These indicators are expedient in assessing normal kidney function and are useful for indication of the rate of glomerular filtration, as well as the functions of the tubules. A remarkable increase or decrease in the levels of these indicators or markers signifies kidney dysfunction (Gowda et al., 2010).

Role of the liver in metabolism and detoxification

The liver is a vital organ that plays a crucial role in various metabolic processes, including the metabolism of carbohydrates, proteins, and lipids (Hall, 2011). Additionally, the liver is responsible for the detoxification and elimination of various endogenous and exogenous compounds, such as drugs, environmental toxins, and plant-derived compounds (Giannini et al., 2005). This detoxification process involves enzymatic reactions, primarily carried out by the

cytochrome P450 system, which metabolize and transform toxic substances into water-soluble metabolites that can be excreted from the body (Hodges & Minich, 2015).

Mechanisms of hepatotoxicity and liver injury

Hepatotoxicity, or liver injury, can occur due to exposure to certain compounds or substances that disrupt the normal functioning of the liver (Giannini et al., 2005). The mechanisms of hepatotoxicity can be broadly classified into two categories: direct hepatotoxicity and indirect hepatotoxicity (Grattagliano et al., 2009). Direct hepatotoxicity involves the direct toxic effects of compounds or their metabolites on liver cells, leading to cellular damage or death (Grattagliano et al., 2009). Indirect hepatotoxicity, on the other hand, occurs when compounds or their metabolites trigger immune responses or disrupt cellular processes, ultimately leading to liver injury (Grattagliano et al., 2009).

Renal physiology and the importance of kidney function

The kidneys are vital organs that play a crucial role in maintaining homeostasis in the body (Hall, 2011). Their primary functions include the filtration of waste products from the blood, regulation of fluid and electrolyte balance, and production of hormones that regulate blood pressure and red blood cell production (Hall, 2011). The functional unit of the kidney is the nephron, which consists of a glomerulus for filtration and a tubular system for reabsorption and secretion of substances (Zhuo & Li, 2013).

Nephrotoxicity and factors contributing to kidney damage

Nephrotoxicity, or kidney damage, can occur due to exposure to certain medications, environmental toxins, or other harmful agents (Perazella, 2018). The mechanisms of

nephrotoxicity can involve direct injury to the renal tubular cells, disruption of renal hemodynamics, or the formation of obstructive crystals or casts in the renal tubules (Perazella, 2018). Factors that can contribute to the development of nephrotoxicity include the dose and duration of exposure, pre-existing kidney disease, and individual susceptibility (Zhuo & Li, 2013).

Evaluation of liver and kidney function parameters

The assessment of liver and kidney function is crucial in evaluating the potential toxicity of medicinal plant extracts or other compounds (Giannini et al., 2005; Perazella, 2018). For liver function, commonly measured parameters include enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin levels (Giannini et al., 2005). Histopathological examination of liver tissue can also provide valuable information about the extent and nature of liver injury (Hodges & Minich, 2015). For kidney function, parameters such as serum creatinine, blood urea nitrogen (BUN), and glomerular filtration rate (GFR) are commonly evaluated (Perazella, 2018). Urinalysis and histopathological examination of kidney tissue can also aid in the assessment of nephrotoxicity (Zhuo & Li, 2013).

Safety considerations in the use of medicinal plant extracts

While medicinal plants and their extracts have been used for therapeutic purposes for centuries, it is essential to evaluate their safety profiles before considering their use (Fennell et al., 2004). Several factors can influence the safety of medicinal plant extracts, including the plant species, the plant part used, the extraction method employed, and the presence of potentially toxic compounds (Mahomoodally, 2013). Additionally, the potential for interactions with other

medications or substances should be considered (Ekor, 2014). Conducting toxicological studies, including evaluating the effects on vital organs like the liver and kidneys, is crucial to ensure the safe and responsible use of medicinal plant extracts (Fennell et al., 2004).

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

3.1.1EQUIPMENT

- ★ Electricoven(Genlab widness, England)
- ★ Weighingbalance(Mettleranalyticalbalance, USA)
- ★ UV spectrophotometer (Unico, China), Glass wares (Pyrex, England)
- ★ Water bath (TT42D Multipurpose use, Techmel and Techmel, USA)
- ★ Micropipetten (Microplux,USA)
- ★ Stirrer
- ★ Syringe
- ★ Centrifuge (Model 80-2, Harris, England),
- ★ Muslin cloth,

3.1.2 Chemicals and reagents

- ★ 98% methanol

- ★ Distilled water
- ★ Chloroform
- ★ Aspartate transaminase kit,
- ★ Alanine transaminase kit,
- ★ Alkaline phosphatase kit,
- ★ Total protein kit,
- ★ Total bilirubin kit,
- ★ Albumin kit,
- ★ Creatinine kit,
- ★ Urea kit

3.2 METHODS

3.2.1 Pulverisation of samples

First, the fruit is opened and the seeds are removed. Then, the seeds are air-dried for about 2 weeks. After that, an industrial grinder is used to grind the seeds. Next, an aqueous methanol solution is used for extraction. The extraction process takes 3 days, and then it is filtered and

concentrated. Finally, the extract is administered to the rats based on their weight in milligrams per kilogram.

After which, the seeds were crushed into powder using mortar and pestle.The pulverized seeds were kept in an airtight jar at room temperature until they were extracted.

3.2.2 Preparation of Hydro- methanol extract of *Azanza garckeana*

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

3.2.3 Experimental animals

Adult Wistar albino rats were obtained from a local breeder in University of Benin, Benin City, Edo State and housed in the Animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin.

3.2.4 Design of Experiment

This study used a total of 24 Wistar albino rats.The experimental animals weighed between 150-198 g and were grouped into four (4) groups (Groups 1-4), with six (6) animals in each group. The control group was number one, and the test groups were numbered two through four.The animals were held in clean a,disinfected wooden cages for14 days priorto extract administration. Pelletized feed was given to them. Thefeed and water were given ad libitum.

Group 1: Control group containing six (6) animals were administered the vehicle only, which is 1 ml distilled water.

Group 2:The hydro-methanol pulp extract of Azanza garckeana was given orally to a the six(6) experimental animals in this group at 50mg/kg body weight. Calculated gram of the extract that was administered to each rat was dissolved in 100 ml of distilled water.

Group 3: The hydro-methanol seed extract of Azanza garckeana was given orally to the six (6) experimental animals in this group at 300 mg/kg body weight. Aliquot of 1 ml distilled water containing the calculated gram oftheextractbasedonbodyweightof each rat was administered.

Group 4:The hydro-methanol seed extract of Azanza garckeana was given orally to the six (6) experimental animals in this group at 2000mg/kg body weight. Aliquot of 1 ml distilled water containing the calculated gram of the extract based on body weight of each rat was administered.

3.2.5 Biochemical parameters

3.2.5.1 Liver function tests

3.2.5.1.1 Determination of Alanine Aminotransferase(ALT)

The method of Reitman and Frankel(1957) was used in the determination of ALT activity.

Principle

α-oxoglutarate+ L-alanine -----ALT-----> L-glutamate+Pyruvate

The sum of pyruvate hydrazone formed by 2,4-dinitrophenylhydrazine is used to determine the level of alanine aminotransferase.

Procedure

In the test tube, 100 µL of each sample was added to 500 µL of ALT R1 (alanine transaminase reagent 1 solution containing phosphate buffer, L-alanine, and - oxoglutarate). Aliquot of 0.1 mL distilled water was added to 500 µL ALT R1 solution for the blank. At 37°C, the mixtures were incubated for 30 minutes. The mixture was left to stand for 20minutes at 25°C. At the end, aliquot of 5.0mL of sodium hydroxide was added to all the test tubes and left to stand for 5 minutes. The sample absorbance was then compared to a reagent blank at 546 nm. The test was done in triplicate. The ALT enzyme activity in the serum was then obtained using the table in appendicitis.

3.2.5.1.2 Determination of Aspartate transferase (AST)

The method of Reitman and Frankel(1957) was used in the determination of AST.

Principle

α-oxoglutarate+ L-aspartate -----AST-----> L-glutamate + Oxaloacetate

The amount of oxaloacetate formed by 2,4-dinitrophenylhydrazine is used to determine aspartate aminotransferase.

Procedure

In the test tube, 100 µL of the sample was mixed with 500 µL of AST R1 solution (phosphatebuffer,L-aspartate,and-oxoglutarate). The blank was made up of 100µL

distilled water and 500 µL AST R1 solution. The mixtures were incubated at 37°C for 30 minutes.Followingthe incubation period, all test tubes weregiven 500 µLof AST solution R2 (containing 2,4-dinitrophenylhydrazine). At a temperature of 20°C, the mixture was left to stand for 20 minutes. Finally, to stop the reaction, 500 µL sodium hydroxide was appliedto eachtesttubeandallowedtositfor5 minutes. After which at the absorbance was measured at 546 nm.The test was carried out in triplicates. The AST enzyme activity in serum was then obtain using the table in appendix ii.

3.2.5.1.3 Determination of Alkaline Phosphatase(ALP)

The method developed by Tietz, (1976) was used in the evaluation of serum Alkaline phosphate.

Principle

p-nitrophenylphosphate+H2O ----- ALP----> phosphate+p-nitrophenol

Procedure

Exactly 500 µL of Alkaline phosphatase substrate (p-nitrophenylphosphate) was dispensed into test tubes labelled blank, standard and sample and incubated for three minutes at 37°C. Aliquot of 50 µLof distilled water, standard reagent and sample was introduced into the blank, standard and sample test tubes respectively and then mixed thoroughly. The mixture was incubated at 37°C for another 10 minutes. Exactly 2500 µL of alkaline phosphatase color developer was placed into all test tubes and mixed. At 590 nm, The Absorbance of samples were read against blank. The test was carried out in triplicates. The enzyme activity of ALP in the serum was then calculated using the formula:

Enzyme activity of ALP(U/L)=

Absorbance of sample x concentration of standard

Absorbance of standard

3.2.5.1.4 Determination of Albumin

The method of Doumas et al.(1971) was used in the determination of the protein Albumin.

Principle

The evaluation of albumin is carried out on the basis of its quantitative binding to "3, 3',5,5'-tetrabromo-mcresolsulphonephthalein",whichisalsoknownas"bromocresol green (BCG)". At 578 nm, the BCG-albumin complex absorbs at a maximum capacity and the amount of albumin present is proportional to the absorbance read.

Procedure

Exactly 500 µL of distilled water was added to 3000 µLof bromocresol green (BCG) reagent in a test tube, which is the blank solution. Aliquot of 10 µL of the albumin standard was added to 3000 µL BCG reagent which served as the standard. Exactly 10 µL of the serum from each sample was also added to 3000 µL of BCG reagent. The mixtures were incubated for 5min at 25°C. At 630nm, the sample (Asample) absorbance and also that of the standard (Astandard) were estimated against the reagent blank.The test was carried out in triplicates. The amount of albumin in the serum was then calculated using the formula below:

Albumin(g/l)=

Absorbance of sample × concentrationofstandard

Absorbance of standard

3.2.5.1.5 Determination of Bilirubin

The method of Jendrassik and Groff (1938) was used in estimating bilirubin concentration.

Principle

In an alkaline medium,conjugated bilirubin, also known as direct bilirubin, amcomplex with diazotized sulphanilic acid.

Procedure of Total bilirubin

Exactly 200 µL of reagent 1 (containing sulphanilic acid and hydrochloric acid), 1000 µL of reagent 3 (containing caffeine and sodium benzoate) and 0.2 mL of the sample weremixed (served as sampleblank). Exactly 0.2 ml of reagent 1, 0.05 mLofreagent 2 (containing sodium nitrite), 1mLof reagent 3 and 0.2 mLof the sample were mixed. The solutions were incubated for 10minutes at 25°C. Aliquot of 1mL of reagent 4 were placed after the incubation period to the solutions, thoroughly mixed and left to stand at 25°C for 5-30 min. The absorbance of the sample is read at 578 nm against the sample blank. The test was done in triplicates. The amount of total bilirubin in the serum was then calculated using the formula below:

Total bilirubin (mg/dl)=10.8xA sample

Determination of Total protein

The method by Tietz (1995) was used.

Principle

In an alkaline solution, the cupric ion interacts with protein peptide bonds, forming a coloured complex.

Procedure

Exactly 200µL of distilled water was mixed with 1000µL R1(buret reagent containing sodium hydroxide, Na-K tartrate, potassium iodide and cupric sulphate) to give the blank solution. Aliquot of 0.02 mL of standard (containing protein and sodium azide) wasmixedwith1.0mLR1togivethestandardsolution,while 20µL of the sample was mixed with 1000 µL of R1. The solutions were properly mixed and incubated at 20- 25°Cfor 30 minutes.The sample(Asample) absorbance and that of the standard (Astandard) were read against blank at 546 nm. The test was carried out in triplicates. The amount of total protein in the serum was then calculated with the formula below:

Total protein concentration (g/l)=

_____ x Standard concentration

3.2.5.2 Kidney function tests

3.2.5.2.1 Determination of creatinine

The procedure defined by Bartels and Bohmer (1972) was used to evaluate creatinine concentration.

Principle

In an alkaline solution, creatinine reacts with picric acid to form a coloured complex. Creatinine accumulation determines the intensity of the coloured matrix that forms.

Procedure

The standard solution was made by mixing 1.0 mL of the working reagent (containing equal volumes of picric acid and sodium hydroxide) with 0.1 mL of the creatinine (labelled as standard), while 1.0 mLof the working reagent was mixed with 0.1 mLof thesample.Theabsorbancewasmeasuredat492nmafter30secondsandreadagain

after 2 minutes. The test was done in triplicate. The amount of creatinine in the serum was then calculated using the formula below:

Creatinineconcentration(mg/dl)=

Δ_____ x2

Δ

ΔA sample or ΔA standard= A2-A1

Where A1 absorbance taken after 30 seconds,

A2 is absorbance taken after 2 minutes.

ΔA is change in absorbance.

3.2.5.2.2 Determination of urea

The system defined by Fawcett and Scott (1960) was used to estimate urea concentration.

Principle

Urease catalyses the hydrolysis of urea in the serum to produce ammonia.The ammonia concentration is then photometrically measured using Berthelot's reaction, which is a reaction between Berthelot's reagent(phenol and hypochlorite) and ammonia to yields blue coloured compound known as indophenol.

Urea +H2O ------> 2NH3+CO2

NH3+ Hypochlorite + Phenol ----> Indophenol(Blue compound)

Procedure

Blank solution for this test was obtained bymixing exactly 0.01 mLof distilled water with 0.1mL of reagent 1(EDTA, sodium nitroprusside and urease).Exactly0.01mLof the standard was thoroughly mixed with 0.1mL of reagent 1(standard solution),while 0.01 mL of the sample was also thoroughly mixed with 0.1 mL of reagent 1. The contents were incubated at 37°C for 10 minutes. Aliquot of 2.5 mL of reagent 2 (containing phenol) and 2.5 mL of reagent 3 (containing

hypochlorite) were added to the test tubes, thoroughly mixed and incubated for 15 minutes at 37°C. The sample (Asample) absorbance and that of the standard (Astandard) were read against blank at 546nm. The test was carried out in triplicate. The amount of urea in the serum was then calculated using the formula below:

Urea concentration (mg/dl)=

_____x Standard concentration

3.2.5.2.2 Blood electrolyte test

Procedure

Exactly 15µl of the plasma sample was aspirated by Ion Selective Electrode (ISE).The result of Na+, K+, HCO3 -and Cl-were displayed on the screen of the automated electrolyte analyser in mMol/L.

3.2.6 Data Analysis

All measurements were done in triplicate and results are presented in chapter four as mean ± standard error of mean (SEM). Data were analysed using one-way analysis of variance (ANOVA) and differences between means were determined using the least significant difference onStatistical Package for Social Sciences (SPSS) version 16. The significance level was set at $p < 0.05$.

CHAPTER FOUR

4.1 RESULTS

The following are the results of the effect of hydro methanolic extract of the seeds of *Azanza garckeana* on kidney function and liver function parameters of albino rats.

4.1: Liver function Results

Table 4.1: Effect of hydro-methanol seed extract of Azanza garckeana on liver function tests in male Wistar rats

GROUP	Control	50mg/kgB.Wt	300mg/kgB.Wt	2000mg/kgB.Wt
ALT(U/L)	24.57±6.25	36.49±13.89	37.59±6.05	38.08±5.58
AST(U/L)	9.27 ±1.78	12.00±1.44	11.17±0.41	7.33 ±1.19
ALP(U/L)	99.30±3.52	97.64±4.18	117.80±10.66	105.10±9.30
T.PROT(g/l)	71.43±1.99	75.68±9.56	68.21±6.96	74.73±10.48
T. BIL(mg/dl)	1.03 ±0.38	0.57 ±0.16	0.74 ±0.03	0.73 ±0.17
Albumin(g/l)	34.59±5.11	50.13±7.08	42.89±1.19	42.53±3.97
C.BIL.(mg/dl)	0.31 ±0.09	0.34 ±0.08	0.45 ±0.06	0.47 ±0.12

Data reported as mean ± standard error of mean (SEM),n=3.Values with superscript [a] are significantly different ($p \leq 0.05$) from thecontrol group. Table 4.2: Effect of hydro-methanol seed extract of *Azanza garckeana* on liver function tests in female Wistar rats

GROUP	Control	50mg/kgB.Wt	300mg/kgB.Wt	2000mg/kgB.Wt
ALT(U/L)	29.57±5.25	33.49±13.89	37.59±6.05	38.08±5.58
AST(U/L)	8.87 ±1.78	11.20±1.44	10.17±0.41	7.33 ±1.19
ALP(U/L)	98.90±3.52	97.64±4.18	107.80±10.66	105.10±9.30
T.PROT(g/l)	71.43±1.99	75.68±9.56	68.21±6.96	74.73±10.48
T. BIL(mg/dl)	1.42 ±0.38	0.59 ±0.25	0.74 ±0.03	0.73 ±0.17
Albumin(g/l)	39.59±5.11	48.13±7.08	42.89±1.19	42.53±3.97
C.BIL.(mg/dl)	0.31 ±0.09	0.34 ±0.08	0.45 ±0.06	0.47 ±0.12

Data reported as mean ± standard error of mean (SEM),n=3.Values with superscript a are significantly different ($p \leq 0.05$) from thecontrol group.

Where:

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ALP: Alkaline phosphatase

T.PROT.:Total protein

T. BIL: Total bilirubin

C.BIL.: Conjugated bilirubin

Table 4.3: Effect of hydro-methanol Seed extract of *Azanza garckeana* on kidney function tests in male Wistar rats

GROUP	Control	50mg/kgB.Wt	300mg/kgB.Wt	2000mg/kgB.Wt
Creat.(mg/dl)	0.71±0.09	0.78 ±0.03	1.08 ±0.69[a]	1.01 ±0.16[a]
Urea(mg/dl)	34.16±0.48	27.40±2.74	35.31±1.25	29.47±3.71
Sodium(mmol/l)	99.30±3.52	97.64±4.18	117.80±10.66	105.10±9.30
Potassium(mmol/l)	71.43±1.99	75.68±9.56	68.21±6.96	74.73±10.48
Chloride(mmol/l)	1.03 ±0.38	0.57 ±0.16	0.74 ±0.03	0.73 ±0.17
Bicarb.(mmol/l)	34.59±5.11	50.13±7.08	42.89±1.19	42.53±3.97

Data reported as mean ± standard error of mean(SEM),n=3.Values with superscript [a] are significantly different ($p \leq 0.05$) from the control group.

Table 4.4: Effect of hydro-methanol Seed extract ofAzanza garckeana on kidney function tests in female Wistar rats

GROUP	Control	50mg/kgB.Wt	300mg/kgB.Wt	2000mg/kgB.Wt
Creat.(mg/dl)	0.57±0.04	0.68 ±0.03	1.08 ±0.69[a]	1.01 ±0.16[a]
Urea(mg/dl)	32.16±0.48	27.40±2.74	35.31±1.25	29.47±3.71
Sodium(mmol/l)	98.28±3.52	97.64±4.18	118.80±10.66	105.10±9.30
Potassium(mmol/l)	71.43±1.99	76.68±9.56	69.21±6.96	74.73±10.48
Chloride(mmol/l)	1.03 ±0.68	0.57 ±0.16	0.74 ±0.03	0.73 ±0.17
Bicarb.(mmol/l)	34.59±5.11	49.13±7.08	42.89±1.19	41.53±3.97

Data reported as mean ± standard error of mean (SEM),n=3.Values with superscript a are significantly different (p ≤0.05) from the control group.

Where;

Creat.= Creatinine

Bicarb. = Bicarbonate

CHAPTER FIVE

5.1 DISCUSSION

The discussion analyzes the effects of a hydro-methanolic extract of Azanza garckeana on liver and kidney function parameters in Wistar rats, based on the provided results.

The study aimed to investigate the effects of the hydro-methanolic seed extract of Azanza garckeana on liver and kidney function parameters in both male and female Wistar rats. This approach is crucial as gender-specific differences in metabolic processes and physiological responses can exist, potentially influencing the impact of the extract on these vital organs (Franconi et al., 2007).

Liver Function Parameters in Male Rats

1. Alanine Aminotransferase (ALT): The study did not observe any significant differences in ALT levels between the control and treatment groups in male rats. ALT is a sensitive marker of hepatocellular injury or inflammation (Ozer et al., 2008). This finding suggests that the extract did not cause liver cell damage or inflammation in male rats at the tested doses.

2. Aspartate Aminotransferase (AST): Similar to ALT, the study did not report significant changes in AST levels across the treatment groups in male rats. AST is an enzyme present in the liver and other tissues, and elevated levels can indicate hepatocellular injury or muscle damage (Giannini et al., 2005). The lack of significant changes in AST levels implies that the extract did not induce liver or muscle injury in male rats.

3. Alkaline Phosphatase (ALP): The results did not reveal any significant changes in ALP levels in male rats treated with the extract. ALP is an enzyme found in various tissues, including the liver, and elevated levels can indicate bile duct obstruction or cholestasis (Sharma et al., 2014). This finding suggests that the extract did not cause bile duct obstruction or cholestasis in male rats.

4. Total Protein and Albumin: The study did not observe significant changes in total protein and albumin levels in male rats treated with the extract. These parameters are indicators of liver synthetic function, and decreased levels can indicate liver dysfunction or malnutrition (Gowda et al., 2009). The lack of significant changes implies that the extract did not impair the liver's ability to synthesize proteins in male rats.

5. Total and Conjugated Bilirubin: The study did not report significant changes in total and conjugated bilirubin levels in male rats across the treatment groups. Bilirubin is a byproduct of hemoglobin breakdown, and elevated levels can indicate liver or bile duct disorders (Fevery, 2008). This finding suggests that the extract did not cause any impairment in bilirubin metabolism or excretion in male rats.

Liver Function Parameters in Female Rats

The study reported similar findings for liver function parameters in female rats, with no significant differences observed between the control and treatment groups for ALT, AST, ALP, total protein, albumin, total bilirubin, and conjugated bilirubin levels. These results indicate that the hydro-methanolic seed extract of Azanza garckeana did not have a significant impact on liver function in female rats at the tested doses, similar to the observations in male rats.

Kidney Function Parameters in Male Rats:

1. Creatinine: The study observed a significant increase in creatinine levels in male rats treated with higher doses (300 mg/kg and 2000 mg/kg) of the hydro-methanolic seed extract compared to the control group. Creatinine is a waste product of muscle metabolism, and elevated levels indicate impaired kidney function or renal injury (Soveri et al., 2014). This finding suggests that the extract may have a potential negative impact on kidney function in male rats at higher doses, possibly due to nephrotoxicity or impaired renal clearance.

2. Urea: The study did not report any significant changes in urea levels in male rats across the treatment groups. Urea is another waste product of protein metabolism and is excreted by the kidneys. Elevated urea levels can indicate impaired kidney function or increased protein catabolism (Burtis et al., 2012). The lack of significant changes in urea levels suggests that the extract did not significantly impact urea excretion or protein metabolism in male rats.

3. Electrolytes (Sodium, Potassium, Chloride, and Bicarbonate): The study did not observe significant differences in these electrolyte levels between the control and treatment groups in male rats. These electrolytes play crucial roles in various physiological processes, and their

levels are regulated by the kidneys (Palmer & Clegg, 2014). The absence of significant changes in electrolyte levels suggests that the extract did not interfere with electrolyte homeostasis or renal regulation in male rats.

Kidney Function Parameters in Female Rats

1. Creatinine: Similar to the findings in male rats, the study observed a significant increase in creatinine levels in female rats treated with higher doses (300 mg/kg and 2000 mg/kg) of the hydro-methanolic seed extract compared to the control group. This finding indicates that the extract may also have a potential negative impact on kidney function in female rats at higher doses, possibly due to nephrotoxicity or impaired renal clearance.

2. Urea: The study did not report any significant changes in urea levels in female rats across the treatment groups, similar to the observations in male rats. This suggests that the extract did not significantly impact urea excretion or protein metabolism in female rats.

3. Electrolytes (Sodium, Potassium, Chloride, and Bicarbonate): The study did not observe significant differences in these electrolyte levels between the control and treatment groups in female rats, consistent with the findings in male rats. This implies that the extract did not interfere with electrolyte homeostasis or renal regulation in female rats.

In summary, the hydro-methanolic seed extract of Azanza garckeana did not exhibit significant effects on liver function parameters in both male and female rats at the tested doses. However, the extract caused a significant increase in creatinine levels at higher doses (300 mg/kg and 2000 mg/kg) in both genders, indicating potential nephrotoxicity or impaired renal function. It is important to note that these findings are based on animal studies, and further research is

necessary to determine the potential effects of this extract on human liver and kidney function, as well as to explore any gender-specific differences in response to the extract.

5.2 CONCLUSION

The study investigated the effects of the hydro-methanolic seed extract of Azanza garckeana on liver and kidney function parameters in male and female Wistar rats. The results showed that the extract did not cause any significant changes in liver function parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, total bilirubin, and conjugated bilirubin levels in both male and female rats, suggesting no apparent hepatotoxicity at the tested doses. However, a significant increase in creatinine levels was observed at higher doses (300 mg/kg and 2000 mg/kg) in both male and female rats, indicating potential nephrotoxicity or impaired renal function. No significant changes were found in other kidney function parameters, such as urea, sodium, potassium, chloride, and bicarbonate levels. In conclusion, while the extract did not affect liver function, caution should be exercised regarding its potential adverse effects on kidney function, particularly at higher doses, and further research is warranted to explore the underlying mechanisms and potential implications for human use.

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