TOXICITY STUDIES ON ALUMINUM OXIDE (Al_2O_3) NANOPARTICLES IN TESTIS AND EPIDIDYMIS OF MALE WISTAR RAT

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BENIN CITY

EDO STATE, NIGERIA

APRIL, 2024

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AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

APRIL, 2024

CERTIFICATION

This is to certify that this project titled "**Toxicity Studies on Aluminum Oxide (Al_2O_3) Nanoparticles in testis and epididymis of male wistar rat**" was carried out by **"Sarah Edeoghogho ALIMIMIAN"** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University Of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award Of Bachelor of Science degree in Environmental Management and Toxicology.

…….. ……

Dr (Mrs) O.A. Edene Date

Project Supervisor

…… ……

Prof. A. A. Enuneku Date

Head of Department

DECLARATION

I **"SARAH EDEOGHOGHO ALIMIMIAN"** declare that "**TOXICITY STUDIES ON ALUMINUM OXIDE (Al_2O_3) NANOPARTICLES IN TESTIS AND EPIDIDYMIS OF MALE WISTAR RAT"** *is* my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

…… ……

Sarah Edeoghogho Alimimian Date

DEDICATION

This work is dedicated to God almighty for the guidance, strength and protection and also to my mum Mrs Helen Alimimian for her unconditional love and provision. I also dedicate this project to my aunt and her husband Mr and Mrs Thomas Akpasubi for their love and support all through my years in school. I would also like to dedicate this project to Mr Osasu Idehen who became a father to me, with his unconditional love and support.

ACKNOWLEDGEMENTS

I am deeply grateful to God Almighty for granting me life, strength, and good health to undertake this study. I extend my heartfelt appreciation to my project supervisor, Dr. (Mrs.) O.A. Edene, for her patience, valuable suggestions, and guidance throughout this study. I would also like to thank my Head of Department, Prof. Alex Enuneku, for creating a conducive environment for the successful completion of this research.

My sincere gratitude goes to the entire staff of the Department of Environmental Management and Toxicology for their tireless efforts in imparting knowledge to me. I am thankful to my supportive siblings, friends, including Annabel, Uche, Kuma, Silver, Pamela, and Shegun, as well as my project teammates, for their unwavering support throughout this project.

I appreciate everyone for their invaluable contributions, which have played a significant role in the successful completion of this project work.

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ABSTRACT

In the realm of nanotechnology, the utilization of nanoparticles has surged across diverse industries, ranging from electronics to medicine. Among these nanoparticles, aluminum oxide nanoparticles (Al_2O_3 – NPs) have gained significant attention due to their unique physicochemical properties. However, concerns regarding their potential toxicity have prompted extensive research into identifying strategies to mitigate their adverse effects. This study aimed to investigate the effects of Al_2O_3 nanoparticles on testis and epididymis of male wistar rat. The effects were investigated using hormonal assay, and standard methods to determine oxidative stress markers. In the current investigation, male wistar rats were given intraperitoneal doses of (Al_2O_3) 30 mg/kg, 50 mg/kg, 70 mg/kg, 100 mg/kg body weight over a period of seven (7) days. The result obtained showed the detrimental impact of Al_2O_3 – NPs on reproductive health. It suggests that these harmful effects are closely linked to the nanoparticles' chemical composition, size, dosage, and duration of exposure. Specifically, the study highlights that Al_2O_3 – NPs can disrupt the production of sex hormones, leading to adverse effects on sperm quality and potentially resulting in infertility. Given the harmful effects of aluminum oxide nanoparticles, the use of antioxidants is proposed as a measure to reduce potential risks associated with Al_2O_3 – NP toxicity on the testis and epididymis of male wistar rats.

CHAPTER ONE

1.0 INTRODUCTION

In the field of nanotechnology, the application of nanoparticles has increased in a variety of industries, including electronics and medical. Due to their special physicochemical characteristics, aluminum oxide nanoparticles (Al_2O_3 NPs) have drawn the most attention among these nanoparticles. However, a lot of research has been done because of worries about their possible toxicological consequences, especially on reproductive organs. Examining the possible effects of Al_2O_3 NPs on the testis and epididymis is crucial since these organs are essential to the male reproductive system and its ability to support the species. As nanotechnology continues to advance, the increased exposure to engineered nanoparticles raises questions about their safety, prompting researchers to delve into comprehensive toxicity studies. This study focuses on the male wistar rat as the experimental model, acknowledging its relevance in translational research due to its physiological similarities to humans. This research endeavors to elucidate the intricate interactions between aluminum oxide nanoparticles and the testis and epididymis of male wistar rats. Through a multidisciplinary approach, encompassing histopathological, biochemical, and molecular analyses, we aim to unravel the potential adverse effects, if any, on spermatogenesis, hormone regulation, and overall reproductive health. The outcomes of this study are anticipated to contribute valuable insights to the existing knowledge base, fostering informed decision-making in the development and application of aluminum oxide nanoparticles while ensuring the preservation of male reproductive health.

1.1 Background of study

Nanoparticles are tiny materials with sizes ranging from 1 to 100 nm. Based on their characteristics, forms, or sizes, they can be divided into several classes. Fullerenes, metal nanoparticles, ceramic nanoparticles, and polymeric nanoparticles are among the several

groups. NPs' large surface area and Nano scale size give them special physical and chemical characteristics (Khan and Saeed, 2017). Twenty percent of all the atoms in each 10-nm-diameter silica nanosphere are found at the surface; a teaspoon, or around 6 ml, of these nanospheres has a surface area greater than twelve doubles-sized tennis courts. The three primary physical characteristics of nanoparticles are as follows: they are very mobile in the free state (for example, a 10-nm-diameter silica nanosphere in water will sediment at a rate of 0.01 mm/day under gravity if no other external influence is present); they may also display what are called quantum effects. Because of this, depending on the product or application, the composition of nanoparticles changes substantially (Jarvie, 2019). However, there was a growing concern about the potential adverse effects of nanoparticles on human and environmental health, especially on the reproductive system (Wu *et al.,* 2019). NPs may have negative effects on the development and reproductive organ function, physiological structure, germ cells, and fertility in humans and animals (Brohi *et al.,* 2017). By destroying sertoli cells, leydig cells, and germ cells, NP accumulation harms the testis, epididymis, ovary, and uterus. This results in reproductive organ dysfunction, which negatively impacts sperm quality, quantity, morphology, and motility or lowers the number of mature oocytes and interferes with primary and secondary follicular development. Furthermore, NPs have the ability to alter released hormone levels, which can modify sexual behavior. (Wang *et al.,* 2018). Among the different types of nanoparticles, metal oxide nanoparticles such as aluminum oxide (Al_2O_3) and zinc oxide (ZnO) are commonly used in various products and processes (Burklew *et al.,* 2012)

Al_2O_3 NPs, or aluminum oxide nanoparticles, are a kind of metal oxide nanomaterials that have a high surface area, mechanical strength, and a porous, economical corundum-like structure. These nanoparticles are useful in a variety of applications because they are readily handled, accessible, and show remarkable chemical stability under harsh conditions

(Hassanpour *et al.,* 2018). Al_2O_3 – NPs find widespread applications in various aspects of daily life, such as altering polymers, enhancing textiles, aerospace, electronics, pharmaceuticals, cosmetics, heat transfer fluids, wastewater treatment, biosensors, biofiltration, drug delivery, and serving as carriers for immunization antigens (Hamdi, 2020). Aluminum oxide nanoparticles (Al_2O_3 – NPs) are generally found in skin care products, such as cosmetics and direct or oral exposure to Al_2O_3 – NPs may cause genotoxic effects (Yousef *et al.,* 2022). Exposure to Al_2O_3 – NPs may produce reactive oxygen species (ROS) within the cells and impair the level of antioxidant activities (Yousef *et al.,* 2022). Because of their numerous technical applications, aluminum oxide nanoparticles (Al_2O_3 – NPs) are one of the most popular nanosized materials in the US market. Concerns regarding their propensity to pollute the environment have been raised by their extensive commercial use. Al_2O_3 – NPs have been linked to genotoxicity. Reactive oxygen species (ROS) may be a mediating factor in the pro-inflammatory actions that lead to this genotoxicity. (Yousef *et al.,* 2019).

The use of animal experimentation is instrumental in gaining insights into human biology. Rodents, particularly rats, are frequently employed in these experiments and are among the most commonly utilized animals for this purpose (Ghasemi *et al.,* 2021). The specific organism of interest in this study is the male wistar rat, an albino rat breed, is widely utilized in laboratory research due to its distinctive features, including a broad head, elongated ears, and a tail length consistently shorter than its body length. Renowned for its high activity level, the wistar rat is a preferred choice in laboratory settings compared to other breeds such as the Sprague Dawley (Health Soothe, 2022). Rats are used for physiological and pharmacological research when they are between the ages of two and five months. Because of their age, adult rats have varying physiological states, which might affect the results of experiments on them (Sudakov *et al.,* 2021).

The specific organs of interest in this study are the testis and the epididymis. The testis is the most important organ for reproductive and sexual function (Ilacqua *et al.,* 2018). In animals, with the exception of primitive species, the formation of male reproductive cells, known as spermatogenesis, takes place in specialized organs called testes. In mammals, these organs consist of intricate structures called convoluted seminiferous tubules. Within the spermatogenic epithelium lining these tubules, germ cells undergo development (Kulibin and Malolina, 2021). The testes, epididymis, vas deferens, and prostate are the internal organs of the male reproductive system; the scrotum and penis are the exterior structures. These highly vascularized structures have several glands and ducts that aid in the production of vital androgens for male development as well as the generation, storing, and ejaculation of sperm for conception (Purnima and Ishwarlal, 2019). The male reproductive system's duties include producing androgens, such as testosterone, that support spermatogenesis and facilitate the transfer of sperm into the female reproductive system for fertilization. Because they are in charge of producing and transporting sperm as well as androgens, the testes function as both endocrine and exocrine organs (Purnima and Ishwarlal, 2019).

The epididymis is a tube-shaped organ that connects the vas deferens to the testis. It is divided into four sections: the caput, corpus, cauda, and the first segment. As sperm pass through the epididymis and interact with the unique luminal conditions in each area, the process of sperm maturation occurs (James *et al.,* 2020). The vital function of the epididymis as a vital reproductive organ, in charge of processes like sperm concentration, maturation (including the development of sperm motility and fertilizing ability), and sperm storage and protection (James *et al.,* 2020).

Al_2O_3-NPs have been shown in multiple studies to be able to cross biological barriers and settle in various body tissues and organs. However, when they build up in the body, they can cause a variety of negative effects, including cytotoxicity, inflammatory response,

carcinogenicity, genotoxicity, mitochondrial dysfunction, ROS generation, neurotoxicity, and hepato- and nephrotoxicity. Consequently, it is important to look at the possible hazards to reproductive health, particularly for people who are exposed to Al_2O_3-NPs at work. (Hamdi, 2020).

1.2 Statement of problem

Concerns over nanoparticles' possible effects on human health have been raised by their increasing use in a number of industries. Commonly found in consumer goods, aluminum oxide (Al_2O_3) nanoparticles have special physicochemical characteristics that could interact with biological systems. In the food business, engineered nanomaterials have become more and more common in recent years. They are frequently utilized in juice clarifiers, food additives, storage life sensors, and food packaging. (Gu *et al.,* 2018). Due to nanoparticles' special physicochemical characteristics, which include their small size, strong reactivity, and effective antibacterial qualities (Gu *et al.,* 2018).

Studies have shown that nanoparticles may cross the blood-testis barrier (BTB) and harm the testis (Nasrin *et al.,* 2023). The male reproductive system has a crucial defensive mechanism called BTB that may stop foreign particles from penetrating. Although it is theoretically impossible for nanoparticles to get through the BTB, several studies have shown that they can do so and end up being stored in the testes (Nasrin *et al.,* 2023). The male reproductive organ has been identified to be vulnerable to environmental stress such as external toxicants and even nanoparticles (Tang *et al.,* 2019). The potential of certain nanoparticles (NPs) to stimulate the generation of reactive oxygen species (ROS), cause DNA damage, and trigger apoptosis suggests their potential in antibacterial and cancer treatments. However, concerns about their inherent toxicity, easy entry, and accumulation in organisms raise questions about their medical use, particularly regarding cytotoxic effects on the male reproductive system and male fertility (Pinho *et al.,* 2020). Aluminum oxide nanoparticles may possibly enter the

food chain and be responsible for toxicity in animals (Rastogi *et al.,* 2017). The toxicological impacts of Al_2O_3 – NPs on male reproductive system have limited information. Therefore, it is important to assess the possible testicular toxicity induced by Al_2O_3 – NPs administration (Hamdi, 2020).

Several studies have noted that aluminum oxide nanoparticles (Al_2O_3 – NPs) have the ability to traverse biological barriers and accumulate in various organs and tissues. Nevertheless, the accumulation of these particles at specific body sites may result in undesirable effects, including genotoxicity, inflammatory response, carcinogenicity, cytotoxicity, ROS generation and mitochondrial dysfunction, hepato- and nephrotoxicity (Hamdi, 2020)

Although aluminum oxide nanoparticles have been shown in numerous studies to be able to pass through biological barriers and accumulate in various organs, there is a significant knowledge gap about the precise effects of these particles on the male reproductive system. Few studies have been done on the possible histological and functional changes that aluminum oxide exposure may cause in the testis and epididymis of male wistar rats. To fully evaluate the hazards to reproductive health associated with exposure to aluminum oxide nanoparticles, this gap must be filled.

The current study aims to investigate the toxic effects of different concentrations of grade aluminum oxide on the testis and epididymis of male wistar rat. Specifically, we seek to understand the impact of aluminum oxide exposure on the and functional aspects of the testicular and epididymal tissues, addressing potential concerns related to reproductive health in male wistar rats.

1.3 Justification of study

In recent years, the widespread use of aluminum oxide nanoparticles (Al_2O_3 – NPs) in various industrial and consumer applications has prompted concerns regarding their potential impact on biological systems. Among the myriad of vital organs, the testis and epididymis stand out as crucial components of the male reproductive system. Understanding the effects of aluminum oxide on these organs is not only scientifically pertinent but also holds significant implications for occupational safety, environmental health, and the broader field of nanotoxicology.

While previous studies have delved into the toxicity of nanoparticles, there is little information on the specific effects of aluminum oxide on the male reproductive system. This research seeks to bridge this gap by undertaking an in-depth examination of the testis and epididymis, unraveling the complex intricacies of potential reproductive health implications.

The relevance of this study extends beyond the confines of the laboratory, reaching into occupational and environmental domains. Individuals working in industries where aluminum oxide is prevalent may be at risk of exposure, necessitating a thorough understanding of potential occupational hazards. Moreover, the environmental impact of aluminum oxide nanoparticles must be assessed to safeguard ecosystems. By elucidating the effects on the testis and epididymis, this study contributes to the development of guidelines that promote safe nanoparticle use in occupational settings and inform environmental regulations.

Examining the male reproductive system is important because it serves as a watchdog over the health of the reproductive system as a whole. The intricate process of reproduction

depends on the testis, which is in charge of spermatogenesis, and the epididymis, which is essential for sperm maturation and transit. Any alterations to these procedures could have far-reaching effects and jeopardize male fertility. Thus, it is critical to comprehend how aluminum oxide affects these organs in order to evaluate and reduce any possible dangers to reproductive health that may arise from nanoparticle exposure.

Nanotoxicology, as a field, is continually evolving, and this research contributes to the growing body of knowledge in this domain. By examining both the quantitative and qualitative aspects of reproductive biomarkers, oxidative stress, and hormonal responses.

This study's translational impact is significant. The results may find application in real-world settings, where they could impact the creation of safe nanoparticle usage guidelines, mold environmental regulations, and direct the creation of nanoparticle designs for diverse uses. This study's ethical considerations guarantee the responsible treatment of research subjects, which upholds the integrity of scientific investigation.

1.4 Aims and objectives

1.4.1 Aims

The primary aim of this study is to evaluate the effects of exposure to different concentrations of aluminum oxide on the testis and epididymis of male wistar rats.

1.4.2 Objectives:

The specific objectives include:

1. To assess the functional alterations in the testis and epididymis of male wistar rats following aluminum oxide exposure.

2. To assess the impact of aluminium oxide nanoparticles on oxidative stress parameters (SOD, CAT, MDA) testis and epididymis of male wistar rats.
3. To examine the effects of exposure to different concentrations of Al_2O_3 on hormonal levels in male wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Aluminum oxide nanoparticles

Nanotechnology creates tiny materials called nanoparticles (NPs). These are tiny particles, measuring fewer than 100 nanometers in at least one dimension (Khan, Saeed and Khan, 2019). In the 21st century, nanotechnology has developed as a notable scientific accomplishment. The creation, control, and use of materials smaller than 100 nanometers belong to the interdisciplinary domain of this field. Important applications for nanoparticles can be found in a wide range of fields, such as biotechnology, medical, agriculture, food, and the environment. (Altammar, 2023). Given that NPs migrate from the environment into food, the industry's extensive usage of NPs raises the risk of NP exposure to humans (Chen *et al.,* 2023). Nanoparticles (NPs) exhibit higher toxicity compared to equivalent conventional particles, raising significant concerns about their potential negative impacts on the health of both humans and wildlife (Hamdi, 2020). However, they also have adverse effects on different organs, including the liver, kidneys, and testes (Abdelhameed *et al.,* 2023). Because of their small sizes, nanoparticles can pass through living organism’s physiological barriers and trigger undesirable biological reactions. It is known that nanoparticles can enter the human body through the skin, lungs, or digestive system. They can also induce cardiac issues, lung inflammation, and brain toxicity. (Adjoa *et al.,* 2023). Among the many types of nanoparticles (NPs), aluminum oxide nanoparticles (Al_2O_3 – NPs) hold significant

importance due to their promising technological applications. The widespread use of these NPs in various commercial applications has garnered significant attention, as they are now considered potential environmental pollutants (Yousef *et al.,* 2019).

Nanoparticles of aluminum oxide (Al_2O_3) exhibit a corundum-like structure, wherein each aluminum atom is surrounded by six oxygen atoms. These nanomaterials are readily synthesizable, cost-efficient, and boast exceptional characteristics like an extensive porous surface area, mechanical strength, bio-inertness, and chemical resistance. These qualities make them valuable for biomedical applications (Kumar *et al.,* 2022). Aluminum oxide nanoparticles (Al_2O_3 -NPs) have attracted significant attention to various scientific and industrial fields due to their unique bio−/physicochemical properties: high surface area, high hardness, thermal stability, biocompatibility, surface functionalization, and electrical insulation (Cáceres *et al.,* 2024). Aluminum oxide nanoparticles find application in vaccine production, particularly in the form of phospholipid bilayer-coated nanoparticles. Nevertheless, concerns arise regarding the toxicity of aluminum oxide nanoparticles, as observed post their oral administration through a drug delivery system. These nanoparticles tend to accumulate in various organs including the brain, liver, kidneys, and skin. Additionally, aluminum oxide nanoparticles have been linked to possible hazards such hepatotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity (Abdelhameed *et al.,* 2023). Extensive research on the toxicity of aluminum oxide nanoparticles (Al_2O_3 – NPs) has been conducted both in laboratory settings (in vitro) and within living organisms (in vivo) since 1990. Numerous studies have reported the toxicity of Al_2O_3 nanoparticles to both cancerous and normal cell lines (Grigore *et al.,* 2017). Exposure to NPs may result in critical toxicity during the development of the reproductive system (Wang *et al.,* 2018). Studies revealed that nanoparticle exposure led to declines in semen characteristics and disruptions in reproductive hormones (Testosterone, FSH, LH) and thyroid hormones (TSH, T3, and T4).

This could be attributed to elevated levels of free radicals and nitric oxide in the testes, coupled with a decrease in antioxidants (Yousef *et al.,* 2019).). Assessing body weight and organ weights, such as testes, epididymis, seminal vesicle, and prostate, is crucial for understanding health implications and compound toxicity. Changes in these weights serve as sensitive indicators of toxic effects, as demonstrated in a recent study on the impact of Al_2O_3 – NPs exposure on body and sex organ weights (Yousef *et al.,* 2019). The study found that Al_2O_3 – NPs adversely affect male fertility by decreasing sperm concentration and normal morphology, increasing DNA damage and caspase-3 expression. Elevated levels of malondialdehyde indicated oxidative damage in the testes, with reduced glutathione and catalase levels. Overall, Al_2O_3 – NPs impact sperm quantity and quality through ROS-induced cellular damage in testicular tissue (Hamdi, 2020).

Al_2O_3 -NPs exhibit significant potential as essential constituents in an extensive array of sophisticated materials and uses, encompassing enhanced coatings, energy storage mechanisms, environmental restoration, optoelectronics, photonics, and personal care products. (Cáceres *et al.,* 2024). Aluminum oxide nanoparticles find diverse applications due to their unique properties, making them indispensable in various fields of science and industry.

With the chemical formula Al_2O_3, aluminum oxide, sometimes referred to as alumina, is an amazing substance with a variety of uses. Al_2O_3-NPs are equally accessible and easily controllable as other metal oxide nanoparticles. High surface area, mechanical strength, and exceptional chemical stability are displayed by these commercially feasible nanoparticles in harsh circumstances, such as hot and abrasive settings. They also have a low electrical conductivity. (Hassanpour *et al.,* 2018). Aluminum oxide (Al_2O_3) nanoparticles find diverse uses in both industrial applications and personal care products (Budimir *et al.,* 2020).

These nanoparticles can be easily synthesized at a low cost and exhibit exceptional properties, including a significant porous surface area, robust mechanical strength, bio-inert characteristics, and resistance to chemicals. These attributes make them valuable for various biomedical applications (Kumar *et al.,* 2022). Considering the strategic importance of Al_2O_3 nanoparticles and their extraordinary properties, they have found diverse applications, particularly in biomedicine and biotechnology in which their utility was explored in drug delivery, biosensing, treatment of diseases, destruction of microbes, and biomolecular stabilization (Hassanpour *et al.,* 2018). Aluminum oxide nanoparticles are cost-effective and efficient in removing arsenic from water, endorsed by the United Nations Environmental Program (Kausar *et al.,* 2022). Aluminium oxide (Al_2O_3) nanoparticles are class of metal oxide nanoparticles that have diverse applications owing to their exceptional physicochemical and structural features such as resistance towards wear, chemicals, mechanical stresses as well as their favourable optical properties and a porous vast surface area (Hassanpour *et al.,* 2018). They include: catalysis, biomedical applications, antimicrobial coatings, electronics and optoelectronics, ceramics and composites.

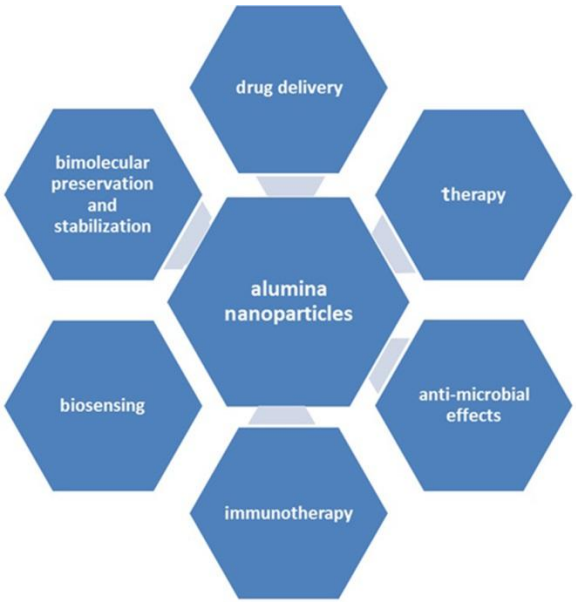

Figure 2.1 Biomedical applications of Al_2O_3 nanoparticles

Source: (Hassanpour *et al.,* 2018)

Alumina (Al_2O_3) nanoparticles are rapidly gaining prominence as a versatile material across numerous industries, showcasing immense potential in various scientific domains. Their emergence is attributed to the compelling chemical and physical properties they exhibit, rendering them highly appealing for extensive research and development endeavors. The unique characteristics of alumina nanoparticles position them as promising subjects for exploration, opening up avenues for innovation and application in diverse sectors. This rising interest is indicative of the broad spectrum of opportunities these nanoparticles offer, signaling their significance in shaping advancements across a range of scientific and industrial realms (Devraj, 2017).

2.2. Physiochemical properties of aluminum oxide nanoparticles

Physicochemical parameters offer insights into how a substance behaves concerning its physical characteristics, chemical reactivity, and interactions with other substances. These parameters are essential for comprehending how a substance will behave, what will happen to it over time, and its potential effects in different environments. In essence, they provide a detailed understanding of a substance's behavior, fate, and impact, which is crucial for informed decision-making in various scientific, industrial, and environmental contexts. Physiochemical parameters include particle size, surface area, chemical composition, purity, zeta potential, crystallinity, porosity, and morphology

Nanosized aluminum oxide, also known as nanosized alumina, can take spherical or fiber forms. Despite a white powder appearance, the nanoparticles pose respiratory and eye irritation risks. With a length-diameter ratio of 20,000,000:1, the highly oriented fibers exhibit weak interaction and lack surface pores, but have concentrated hydroxyl groups. The nanoparticles' physiochemical characteristics depend on their dimension and size, influencing

atomic arrangement. Reports indicate three layers: the surface layer with metal ions or molecules, the distinct shell layer, and the nanoparticle's core representing its chemical formula (Coldwell, 2022).

Aluminum oxide nanoparticles (Al_2O_3 NPs) possess a corundum crystal structure, displaying high hardness, thermal stability, and electrical insulation. Biocompatible and surface-modifiable, they exhibit antimicrobial properties, suitable for medical coatings. Additionally, these NPs enhance catalysis and hold promise in various advanced applications, including coatings, energy storage, environmental remediation, optoelectronics, and personal care products (Rodrigo *et al.,* 2024). The thermal properties of nanoparticles depend on the metals they contain. General knowledge dictates that metals such as copper and aluminum oxides exhibit superior thermal conductivities compared to most non-metallic solids or fluids (Coldwell, 2022).

2.3. Implications of aluminum oxide nanoparticles exposure to human health

Among the most commonly used nanomaterials are aluminum oxide nanoparticles (Al_2O_3 NPs), yet there isn't much information on risk assessment and identification at this time. Because of its low density and resistance to corrosion, this metal finds application in the building, transportation, and aerospace industries. However, the International Agency for Research on Cancer has determined that the manufacture of aluminum is carcinogenic to humans (Kim *et al.,* 2018). The small sizes of nanoparticles give them the ability to permeate physiological barriers of living organisms, causing harmful biological reactions. Nanoparticles are known to enter the human body through the lung, intestinal tract, or skin, and can be toxic to the brain, cause lung inflammation and cardiac problems (Adjoa *et al.,* 2023Aluminum exposure in humans is common and can come from paints, fuel additives, personal care items, medications, and food additives. Aluminum-using businesses and metal refining processes expose workers to occupational hazards. Additionally, aluminum, often in

the stable form of aluminum oxide (Al_2O_3), is present in ultrafine particles in the air. An example of occupational exposure is through sandpaper use, generating inhalable Al_2O_3 dust during polishing (Kim *et al.,* 2018).

Alumina nanoparticles (Al_2O_3 – NPs) are rapidly absorbed and distributed systemically, inducing toxicity through oxidative stress and mitochondrial dysfunction. Their accumulation leads to cellular damage by interacting with cell components, proteins, and genetic materials. Al NPs trigger cell death, disrupt mitochondrial integrity, and generate reactive oxygen species. Previous studies highlight hepato-, nephro-, myocardial, reproductive, and neurotoxic effects, along with inflammatory, apoptotic, and genotoxic impacts (Elkhadrawy *et al.,* 2021). Experimental conditions such as dose, time, and route of exposure, impact the toxicity and distribution of aluminum oxide nanoparticles (Al_2O_3 – NPs). These NPs can linger in the spleen and liver (Alshaimaa *et al.,* 2022). Al_2O_3 – NPs induce reactive oxygen species, pro-inflammatory cytokines, and DNA mutations, causing damage to the brain, liver, kidneys, and immune system. Exposure leads to genetic damage, inflammation, carcinogenicity, cytotoxicity, and mitochondrial dysfunction. Aluminum from oral intake accumulates in bone, kidneys, and brain, potentially causing adverse effects. Al_2O_3 – NPs ' toxicokinetics result in lung accumulation, direct entry into systemic circulation, and distribution to organs, causing histopathological and physiological disorders (Alshaimaa *et al.,* 2022).

2.4. Similarities between rat and humans

While rats and humans may appear vastly dissimilar initially, upon closer examination, numerous similarities emerge between these two species. Both rats and humans belong to the mammalian class, sharing fundamental traits like possessing hair, mammary glands, and the capacity to regulate body temperature. Additionally, they boast intricate anatomical and physiological features, facilitating their adaptation and flourishing within their distinct

habitats. Rats and humans exhibit remarkable parallels in their physiological makeup, dietary requirements, social interactions, and cognitive functions, underscoring their shared evolutionary heritage. From their intricate nervous systems to their four-chambered hearts and skeletal structures, both species display fundamental anatomical similarities. Furthermore, they both necessitate a balanced diet to sustain health and vitality, albeit with variations in digestive complexity (Bonk, 2023)

Socially, rats and humans thrive in group settings, engaging in behaviors that foster social bonds and cooperation. Whether it's through grooming, play, or vocalizations in rat colonies or through familial, communal, and societal interactions among humans, both species demonstrate a need for social connection. Cognitively, rats exhibit impressive memory skills and learning capabilities, while humans possess higher-level cognitive functions such as language and abstract thinking (Bonk, 2023). However, foundational cognitive abilities like attention and problem-solving are evident in both species, indicating shared evolutionary traits. These striking similarities between rats and humans not only offer insights into human physiology and behavior but also highlight the interconnectedness of life forms and the continuum of biological evolution (Bonk, 2023).

Rats and humans frequently experience similar illnesses due to their shared fundamental physiology, analogous organ systems, and comparable body structures. This likeness in biological makeup means that diseases affecting one species can often manifest in similar ways in the other. For instance, both rats and humans possess respiratory, cardiovascular, digestive, and nervous systems with similar functionalities, making them susceptible to analogous health issues. This parallelism in disease susceptibility underscores the evolutionary connections between these species and emphasizes the importance of using rats as model organisms in biomedical research to understand and develop treatments for human ailments (Heimduo, 2023).

The table below show sexual development and age of laboratory rat compared to that of humans (Ghasemi *et al.,* 2021).

Table 2.1 sexual development and age

Developmental stages			Human	Rat
Prenatal	Gestation length		40w	21d
postnatal	Sexual development	Neonatal	0-1m	0-7d
		Infantile	1m-2y	7-21d
		Juvenile/childhood	2-11y	21-35d
		Peripubertal	11-14y	35-55d
		Adolescents	12-18y	55-70d
	Adulthood development	Emerging adulthood	18-25y	70-150d
		Young adulthood	25-40y	150-300d
		Middle adulthood	40-65y	300-600d
		Older adulthood	65-75y	600-730d
		Late adulthood	75+y	730+d
d, postnatal da; w, week; m, month; y, year				

Source: (Ghasemi *et al.,* 2021)

2.5. Zoology of Wistar Rat

Wistar rats are a group of substrains that share a common ancestor. These substrains include both outbred strains, such as Unilever (Wistar WU) and Hannover (Wistar Han), and inbred strains, such Kyoto (Wistar WKY) and Furth (Wistar WF). Outbred stocks are the most commonly used among them in safety evaluation research. (Clements *et al.,* 2022). Adult rats aged 2 to 5 months are commonly used in physiology and pharmacology experiments. However, the impact of age-related changes on experimental outcomes hasn't been thoroughly explored. A study observed a decrease in motor and exploratory activities, along with an increase in anxiety-like behavior as rats aged (Sudakov *et al.,* 2021). These changes varied based on initial behavioral traits. For instance, rats with high motor activity at 2 months showed reduced activity by 5 months, while those with low anxiety levels became more anxious over time (Sudakov *et al.,* 2021). Rats with low activity and high anxiety didn't exhibit significant age-related changes. These findings underscore the importance of considering age when selecting rats for behavioral experiments (Sudakov *et al.,* 2021). Wistar rats have a wide head, they possess long ears, and the length of their tail is always less than their body length (Blaschke, 2022).

The wistar rat (Rattus norvegicus) belongs to the following taxonomic classification (Schoch *et al.,* 2020):

Kingdom: Animalia
Phylum: Chordata
Class: Mammalia
Order: Rodentia
Family: Muridae

Genus: Rattus

Species: Rattus norvegicus

The wistar rat is commonly used in scientific research and is known for its albino white coat color.

2.6. Functions of the Testis

The testes, also known as testicles, constitute a crucial component of the male reproductive system, performing multifaceted functions vital for reproduction and hormonal regulation. These two oval-shaped organs are enclosed within the scrotum, a protective sac of skin positioned outside the body in the anterior pelvic region, proximal to the upper thighs (Boskey, 2021). One of the primary roles of the testes involves the production and storage of spermatozoa until they attain maturity, facilitating their release during ejaculation (Boskey, 2021). This process of spermatogenesis occurs within specialized structures within the testes called seminiferous tubules. These tubules, organized into lobules, undergo intricate cellular processes to generate sperm cells from germ cells, aided by various supporting cells such as sertoli cells (Vaskovic, 2023).

Moreover, the testes serve as endocrine glands, producing and secreting hormones essential for male reproductive function and overall physiological well-being (Boskey, 2021). Chief among these hormones is testosterone, an androgenic hormone pivotal for the regulation of secondary sexual characteristics, including the development of facial and body hair, deepening of the voice, and the growth of the external genitalia during puberty. Additionally, testosterone plays a crucial role in modulating libido, sperm production, and the maintenance of muscle mass and bone density (Vaskovic, 2023). Structurally, the testes derive their characteristic oval shape from the arrangement of lobules composed of coiled seminiferous tubules interspersed with interstitial tissue containing leydig cells. These leydig cells are

responsible for the synthesis and secretion of testosterone in response to luteinizing hormone (LH) stimulation from the pituitary gland (Jewell, 2018).

The testes play a pivotal role in male reproductive physiology, encompassing both gametogenesis and hormone production. Their intricate structure and physiological functions highlight their indispensable role in facilitating male fertility, sexual development, and overall health (Jewell, 2018).

2.7. Functions of the Epididymis

It is widely acknowledged in reproductive biology that the journey of spermatozoa from their origin in the testes to their eventual maturity and functionality involves passage through the epididymis (James *et al.,* 2020). This tubular organ, linking the testis to the vas deferens, is anatomically divided into four distinct regions: the initial segment, caput, corpus, and cauda. Each of these regions contributes uniquely to the process of sperm maturation, imparting essential characteristics necessary for fertilization (Boskey, 2022). During transit through the epididymis, sperm cells undergo a series of transformative processes, including the acquisition of motility and fertilization capabilities. These processes are facilitated by interactions with the specialized luminal microenvironments of each epididymal segment, which provide a milieu conducive to sperm maturation (Boskey, 2022). The epididymis serves as a critical reproductive organ with multifaceted functions essential for male fertility and reproductive success. Apart from facilitating sperm maturation, it plays a pivotal role in sperm concentration, protection against harmful agents, and storage for eventual ejaculation (Shahid, 2022). Of particular interest in recent research is the role of epididymal-derived exosomes, known as epididymosomes, in regulating sperm competency and functionality. These extracellular vesicles are secreted by the epididymal epithelial cells and are believed to carry specific cargo molecules that modulate sperm physiology and function. Their involvement in the communication between epididymal epithelial cells and sperm cells

highlights the intricate interplay between the epididymis and sperm maturation processes (Shahid, 2022).

Furthermore, emerging evidence suggests that understanding the role of the epididymis is not only crucial for elucidating male reproductive physiology but also has implications for offspring health and susceptibility to diseases (James *et al.,* 2020). A comprehensive understanding of the complex interactions within the epididymal microenvironment and its influence on sperm quality and function is essential for advancing reproductive medicine and fertility treatments. The epididymis stands as a vital nexus in male reproductive biology, orchestrating the maturation and functionality of spermatozoa essential for successful fertilization. Continued research into the mechanisms underlying epididymal function and its impact on reproductive outcomes holds promise for improving male fertility assessment, diagnosis, and treatment strategies (James *et al.,* 2020).

2.8. Oxidative stress

An imbalance between the body's capacity to detoxify reactive oxygen species (ROS) and their synthesis causes oxidative stress (Pizzino *et al.,* 2017). While ROS have physiological roles like cell signaling and are by-products of oxygen metabolism, environmental stressors and xenobiotics significantly increase their production, leading to cell and tissue damage (Pizzino *et al.,* 2017). Antioxidants like vitamin E, flavonoids, and polyphenols are utilized to counteract oxidative stress. Interestingly, oxidative stress is also exploited therapeutically, notably in cancer treatment, with some success (Pizzino *et al.,* 2017). The basic idea is that, in an open metabolic system, there is a constant maintenance of a balanced redox state at a specific level, establishing a fundamental redox equilibrium. Any deviation from this equilibrium is perceived as stress, triggering a corresponding stress response (Sies, 2020). The impacts of oxidative stress can be diverse and not always detrimental. For instance, oxidative stress resulting from physical exertion can actually have beneficial effects on the

body's regulation. Exercise induces the formation of free radicals, leading to temporary oxidative stress in muscles. Yet, these free radicals serve to regulate tissue growth and promote the production of antioxidants. Mild oxidative stress may even serve a protective role against infections and diseases. Studies discovered that oxidative stress inhibited the spread of melanoma cancer cells in mice (Eske, 2019). However, prolonged oxidative stress can harm cells, proteins, and DNA, potentially accelerating aging and contributing to the onset of various conditions (Eske, 2019).

The significant impact of inflammation and oxidative stress on male infertility, a global issue with complex causes. It highlights how inflammation, often present without symptoms, is linked to higher levels of proinflammatory agents in the male reproductive system. Oxidative stress, a result of inflammation, damages reproductive cells and components, further impairing fertility. Various factors contribute to male infertility, all ultimately affecting reproductive functions through inflammation and oxidative stress (Dutta *et al.,* 2021). Male infertility often stems from unknown causes, but a significant portion of infertile men exhibit high levels of Reactive Oxygen Species (ROS) in their semen, leading to what's termed "Male Oxidative Stress Infertility (MOSI)." ROS in semen primarily come from two sources: leukocytes and immature sperm. Infections in the male genital tract can trigger additional ROS production by leukocytes, while immature sperm with morphological defects generate intrinsic ROS. When the balance between ROS and antioxidant defense systems is disrupted, oxidative stress (OS) occurs, damaging sperm through processes like lipid peroxidation, sperm DNA fragmentation, and germ cell apoptosis (Dutta *et al.,* 2021).

Free radicals can significantly impair sperm fertility by affecting parameters such as count, motility, and morphology. Oxidative stress induced by free radicals leads to an increase in abnormal sperm production, decrease in sperm count, and fragmentation of sperm DNA, ultimately causing infertility (Asadi, 2017). Exposure to high oxygen pressure reduces sperm

rate and motility, but adding catalase to the culture medium prevents this effect by counteracting the increased production of Hydrogen Peroxide (H_2O_2). Reactive Oxygen Species (ROS), whether produced by leukocytes or sperm, have detrimental effects on sperm function in infertile men. Maintaining a balance between ROS production and elimination is crucial for sperm health and fertility, as sperm are particularly vulnerable to oxidative stress due to their limited cytoplasm, low levels of antioxidants, and high concentration of unsaturated fatty acids. Antioxidant enzymes in sperm may not adequately protect the plasma membrane, making sperm health reliant on antioxidants in seminal plasma. If antioxidants are removed from semen, such as during washing, sperm become susceptible to oxidative damage. Sperm can combat oxidative stress through the antioxidant properties of semen, where ROS are continuously deactivated by seminal antioxidants under normal conditions. Imbalance between ROS production and inactivation by semen antioxidants can lead to oxidative stress conditions, negatively impacting sperm health and fertility (Asadi, 2017).

Oxidative stress harms male fertility by reducing sperm quality and motility, and increasing DNA oxidation (Wu *et al.,* 2020). A study on rats exposed to tert-butyl hydroperoxide (t-BHP) found prolonged effects in the epididymis and sperm, leading to decreased motility and higher DNA oxidation levels in cauda epididymis sperm. These findings highlight the detrimental impact of oxidative stress on sperm quality and male fertility. (Wu *et al.,* 2020). Rats exposed to aluminum oxide nanoparticles (Al_2O_3-NP) showed increased lipid peroxidation (MDA), decreased glutathione (GSH), and catalase (CAT) activities in their testicular tissue, indicating compromised antioxidant defenses and heightened oxidative stress. These findings suggest potential adverse effects on male reproductive health, emphasizing the need for further research. (Hamdi, 2020). In male rats, oral exposure to aluminum oxide nanoparticles (Al_2O_3 – NPs) and zinc oxide nanoparticles (ZnO-NPs), alone or combined, led to significant reproductive toxicity (Yousef *et al.,* 2019). This included DNA fragmentation,

suppressed gene expression, adverse effects on semen characteristics, hormone levels, antioxidant enzymes, and testes histopathology. Increased free radicals, nitric oxide, inflammatory cytokines, and altered gene expression related to mitochondrial function and tumor suppression were observed. The combination of Al_2O_3 -NPs with Zn-ONPs showed higher reproductive toxicity than individual exposures (Yousef *et al.,* 2019)

2.9. Reproductive Hormones

Hormones are organic substances produced by plants and animals, regulating physiological activities and maintaining balance. They prompt specific responses from organs or tissues in tiny amounts (Barrington, 2019). These fascinating chemical messengers play a crucial role in coordinating various functions within your body. Hormones serve as molecular signaling agents orchestrating diverse physiological processes within the human body. Produced and secreted by various glands, organs, and tissues, these biochemical entities collectively constitute the endocrine system (John, 2018). It is a biological substance utilized by multicellular organisms to regulate, synchronize, and govern the activities of their cells and tissues. These compounds have the capacity to regulate a wide array of physiological processes, ranging from metabolism to behavioral responses (Starka and Duskova, 2020).

The key reproductive hormones - estrogen, testosterone, and progesterone - are essential for regulating sexuality and fertility. They control pregnancy, puberty, menstruation, menopause, sex drive, and sperm production. Produced mainly in the ovaries (females) and testes (males), these hormones also affect broader aspects of physiology and behavior. The pituitary gland plays a vital role by producing, storing, and stimulating other reproductive hormones, ensuring the proper functioning of the reproductive system (John, 2018). Understanding reproductive hormones and their functions is crucial for addressing fertility issues. When these hormones are imbalanced or dysfunctional, it can lead to difficulties in conceiving. Advances in medical science, such as assisted reproductive technologies and hormone-based

therapies, allow healthcare providers to intervene effectively by restoring hormonal equilibrium. Ongoing research further enhances our understanding of reproductive endocrinology, enabling early detection and personalized treatment strategies for individuals facing fertility challenges (Starka and Duskova, 2020). The male reproductive system comprises internal and external organs responsible for urinary functions, sexual intercourse, and reproduction. It includes structures involved in producing, maintaining, and transporting sperm cells and semen, which is the fluid surrounding sperm. These organs facilitate the discharge of sperm and the production and secretion of male sex hormones essential for the system's functioning (Gurung *et al.,* 2019).

The male reproductive system comprises of structures essential for sperm production, storage, and ejaculation. Key hormones such as testosterone, synthesized by Leydig cells, regulate male development. Other hormones like inhibin B and Mullerian inhibiting substance (MIS) from Sertoli cells modulate spermatogenesis. Hormonal regulation is orchestrated by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) released by the anterior pituitary, under the influence of gonadotropin-releasing hormone (GnRH) from the hypothalamus, forming the hypothalamic-pituitary-gonadal axis crucial for male sexual function and development (Gurung *et al.,* 2019).

2.9.1Testosterone

Testosterone, the primary male hormone, plays a crucial role in regulating sex differentiation and the development of male reproductive characteristics, sperm production, and fertility (Nassar *et al.,* 2023). During fetal development, both male and female reproductive tissues start off identical. However, in men, the SRY gene on the Y chromosome initiates the production of testicles around the seventh week of gestation. The Sertoli cells produced by these testicles produce a material known as Mullerian-inhibiting substance (MIS) (MacGill, 2023). MIS prompts the regression of female reproductive structures like the Fallopian tubes,

uterus, and upper part of the vagina. Meanwhile, fetal Leydig cells and endothelial cells produce testosterone, which stimulates the differentiation of structures derived from the Wolffian ducts into the male urogenital tract. Testosterone also converts to dihydrotestosterone (DHT) peripherally, promoting the development of the prostate and male external genitalia (Nassar *et al.,* 2023). Additionally, testosterone facilitates the descent of testes through the inguinal canal in the final months of fetal development. In embryos lacking a Y chromosome and the SRY gene, ovaries form instead. These fetal ovaries produce insufficient testosterone, resulting in the absence of Wolffian duct development and MIS. Consequently, female reproductive structures, derived from the Mullerian ducts, develop in these individuals (O'Donnell *et al.,* 2017).

Testosterone, a hormone crucial for male sexual characteristics, is involved in regulating several bodily processes, including sperm production. It is predominantly produced by Leydig cells in the testicles and is considered an androgen. In addition to its role in sexual characteristics and fertility, testosterone influences factors such as libido, bone density, fat distribution, muscle size, strength, and red blood cell production (O'Donnell *et al.,* 2017). Inadequate testosterone levels can lead to infertility due to impaired sperm development. Despite being predominantly associated with males, testosterone also plays a role in women, usually produced in smaller amounts, affecting aspects like libido, bone density, and muscle strength. However, excessive testosterone in women may cause male-pattern baldness and infertility. The brain and pituitary gland regulate testosterone levels, and once produced, the hormone circulates through the bloodstream to carry out its various functions (MacGill, 2023). Testosterone is integral to the regulation of secondary male characteristics, which define masculinity. These characteristics include patterns of hair development peculiar to men, variations in the pitch and depth of voice, and anabolic effects such as increased growth throughout puberty, especially in the skeletal muscles because of higher protein synthesis.

Furthermore, testosterone stimulates erythropoiesis, which causes males to have higher hematocrit levels than females. Reduced testicular size, decreased libido, lower bone density, loss of muscle mass, increased fat buildup, and a fall in erythropoiesis, which may result in anemia, are just a few of the changes that occur as men's testosterone levels decline with age (Nassar *et al.,* 2023).

2.9.2. Follicle-stimulating hormone (FSH)

Follicle-stimulating hormone (FSH) is a hormone secreted by the anterior pituitary gland following stimulation by gonadotropin-releasing hormone (GnRH) released from the hypothalamus. FSH is involved in the processes of sexual development and reproduction in both males and females (Orlowski and Sarao, 2018). Contrary to its name, FSH doesn't directly influence hair growth; instead, it primarily affects the function of ovaries and testicles. It derives its name from its role in stimulating ovarian follicles, which house egg cells.

Hormones act as messengers in the body, conveying instructions to organs and tissues through the bloodstream (Cleveland Clinic, 2023). FSH plays various roles depending on an individual's gender and age. In children, FSH levels are typically low, but as puberty nears, the hypothalamus releases gonadotropin-releasing hormone (GnRH), initiating FSH and luteinizing hormone (LH) production, marking the onset of sexual maturation. In boys, FSH and LH stimulate the testes to produce testosterone, leading to physical changes like body hair growth and sperm production. In girls, these hormones prompt the ovaries to produce estrogen, which facilitates breast development and menstruation. In men, FSH supports sperm production by acting alongside testosterone, which is also triggered by LH. The hypothalamic-pituitary-gonadal axis regulates FSH levels through a feedback system involving various hormones. (Cleveland Clinic, 2023). The hypothalamus releases GnRH, stimulating the pituitary gland to release FSH and LH. These hormones travel through the

bloodstream and bind to receptors in the testes or ovaries, regulating their functions. Hormones released by the gonads influence GnRH production, restarting the cycle. Estrogen in females affects FSH release differently, inhibiting it in the long term but increasing it before ovulation. As part of the feedback loop, male testicular cells secrete inhibin B, which inhibits the secretion of FSH. When this hormone cascade is disturbed, there are insufficient quantities of sex hormone, which affects gonadal function in adults and causes aberrant sexual development in children. (O'Donnell *et al.,* 2017).

Follicle-stimulating hormone (FSH) is structured as a glycoprotein dimer comprising alpha and beta subunits, with the beta subunit being specific to FSH, while the alpha subunit is identical to that found in thyroid-stimulating hormone (TSH), human chorionic gonadotropin (hCG), and luteinizing hormone (LH) (Orlowski and Sarao, 2018). Gonadotropin-releasing hormone (GnRH) initiates the release of FSH. Originating from the hypothalamus, GnRH enters the hypophyseal portal circulation and binds to G-protein-coupled receptors on gonadotropic cells in the anterior pituitary (Brogaard, 2024). These cells then produce and release FSH and LH into the bloodstream. GnRH release occurs in pulsatile fashion, with low-frequency pulses stimulating increased FSH production and high-frequency pulses promoting greater LH production (Brogaard, 2024).

Continuous use of GnRH leads to the suppression of FSH and LH release from the anterior pituitary, resulting in the inhibition of ovulation and estrogen production in women, as observed with drugs like leuprolide. In women, negative feedback from estrogen levels inhibits FSH secretion. Conversely, in men, inhibin B levels, produced by Sertoli cells in response to FSH, suppress FSH secretion via negative feedback (Orlowski and Sarao, 2018).

2.9.3. Luteinizing hormone (LH)

The anterior pituitary gland secretes luteinizing hormone (LH), a complex glycoprotein hormone, in response to the hypothalamus' regular secretion of gonadotropin-releasing hormone (GnRH) (Kazmi and Can, 2021). Both male and female reproductive processes are regulated by LH. LH stimulates the creation of testosterone in males by acting on Leydig cells in the testes, which is essential for male sexual development and features. LH surges in females cause ovarian follicles to break and release mature eggs; they also encourage the follicles to change into the corpus luteum, which secretes progesterone (O'Donnell *et al.,* 2017). Progesterone prepares the uterus for potential embryo implantation during the menstrual cycle. Luteinizing hormone is a key hormone in the reproductive system, regulated by GnRH from the hypothalamus (Kazmi and Can, 2021). Moreover, LH aids in governing the duration and sequence of the menstrual cycle in females by participating in ovulation and the embedding of an egg in the uterus. The Hypothalamic-Pituitary-Gonadal (HPG) axis is an important endocrine route that includes the pituitary gland, the gonads, and the hypothalamus. It regulates the levels of LH in the body. This system makes sure that the blood contains the right amounts of LH (and FSH) by coordinating signals between these three parts. (Ada, 2019)

Firstly, the hypothalamus, located in the brain, releases pulses of Gonadotropin-Releasing Hormone (GnRH). GnRH then stimulates the pituitary gland to produce and release LH. Subsequently, LH travels to the gonads, where it interacts with cells in the ovaries in females and the testes in males. This interaction leads to various effects, including the production of testosterone and estradiol (O'Donnell *et al.,* 2017). The levels of testosterone and estradiol in the bloodstream are continually monitored by the hypothalamus, which adjusts the release of GnRH accordingly. This process, known as the "negative feedback loop," enables the system to self-regulate and maintain LH levels within the appropriate range (Ada, 2019).

Luteinizing hormone (LH) and human chorionic gonadotropin (hCG) are vital hormones involved in the development of both sexes. These hormones play essential roles in various

stages of fetal development and beyond (O'Donnell *et al.,* 2017). LH is responsible for stimulating the production of testosterone in males and regulating ovulation and progesterone production in females. On the other hand, hCG plays a critical role in the early stages of pregnancy by supporting the production of progesterone, maintaining the uterine lining, and signaling the body's readiness for pregnancy. Both LH and hCG levels fluctuate during gestation and are intricately involved in the regulation of reproductive processes in males and females (Nedresky and Singh, 2020). Through the action of a G-protein coupled receptor, luteinizing hormone (LH) stimulates adenylyl cyclase. Cyclic adenosine monophosphate (cAMP) is produced by this enzyme, which raises its concentration within the cell. Protein kinase A (PKA), a kinase molecule, is activated by this increased cAMP. PKA then phosphorylates particular intracellular proteins, which in turn mediates the physiological effects of LH, including ovulation and the synthesis of steroids (Nedresky and Singh, 2020).

2.10 Sperm Analysis

A sperm analysis is a diagnostic procedure that examines a semen sample to assess overall sperm health. During a sperm analysis, several factors are evaluated, including the sperm count per milliliter of semen, sperm morphology (size and shape), and sperm motility (ability to move correctly) (Leonard, 2018). Semen is expelled in discrete portions during ejaculation and comprises a combination of spermatozoa originating from the testes, processed in the epididymides, and mixed with secretions from various male accessory sex organs, such as the prostate, seminal vesicles, bulbourethral glands, and epididymides (Sunder and Leslie, 2020). Assessment of semen quality involves analyzing several parameters, including total sperm count, volume of fluid, sperm concentration, and characteristics of spermatozoa, such as viability, motility, and morphology, along with the composition of secretions. A comprehensive evaluation of these factors is essential for diagnosing male factor infertility (Sunder and Leslie, 2020).

Sperm, the male reproductive cells found in the ejaculate, play a crucial role in fertilization and reproduction. The average sperm count typically falls within the range of 40 million to 300 million sperm per milliliter, although this range can vary slightly among testing centers. By evaluating the sperm count and other parameters, semen analysis provides valuable insights into male fertility and reproductive health (Nall, 2019).

The term "sperm motility" describes a cell's capacity for efficient movement and swimming. Because it allows sperm to pass through the female reproductive system and reach the egg for fertilization, it is essential to male fertility. In order for sperm to successfully conceive, they must be able to go through a variety of settings and barriers. A contributing reason to infertility may be impaired sperm motility, which prevents sperm from reaching and fertilizing the egg. Thus, determining sperm motility is a crucial component of determining male fertility (Dcunha *et al.,* 2020). Fertility depends on sperm motility, which is controlled by intricate structural and molecular signaling pathways. The three primary components of spermatozoa are the neck, which connects the head to the flagellum, the tail, which propels the sperm forward, and the head, which contains nuclear material. Subdivided further into the midpiece, primary piece, and end piece is the flagellum (Dcunha *et al.,* 2020).

Sperm morphology refers to the size and shape of individual sperm cells, crucial for male fertility (Huizen, 2017). Proper morphology is necessary for sperm to effectively fertilize an egg. Evaluating sperm morphology involves assessing characteristics like sperm head shape and tail integrity. Abnormalities in morphology can indicate fertility issues. Factors like genetics and lifestyle influence sperm morphology. Evaluation of morphology is part of a semen analysis to guide infertility treatments and improve pregnancy success rates (Huizen, 2017).

2.10.1. Effects of aluminum oxide nanoparticles on rat sperm

A study assessed sperm quality parameters, including concentration, motility, viability, and abnormalities, in rats exposed to aluminum oxide nanoparticles (Al_2O_3 – NPs). Concentration and motility of sperm were analyzed using the hemocytometer method (Hamdi, 2020). Viability was determined by mixing sperm suspensions with eosin Y, and 1000 sperm were counted to assess abnormalities. The results showed that the concentration, motility, and viability of sperm were significantly lower in the Al_2O_3 – NPs -administrated group compared to the control group. Additionally, there was a significant increase in sperm abnormalities observed in the Al_2O_3 – NPs -administrated group compared to the control group (Hamdi, 2020). Another study's empirical findings delineated within the dataset exhibit a discernible impact of aluminum Oxide nanoparticles (Al_2O_3 – NPs) on male reproductive parameters, notably manifesting in a notable diminution in sperm motility and sperm count, juxtaposed against the control cohort (Yousef *et al.,* 2019). Additionally, a marked elevation in the incidence of abnormal sperm morphology was observed in response to exposure to Al_2O_3 – NPs. These observations underscore the deleterious effects of Al_2O_3 – NPs on male fertility indices, indicative of a perturbation in the intricate physiological processes governing spermatogenesis and sperm function (Yousef *et al.,* 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study Area

The research was conducted at the Anatomy Department's Animal House at the University of Benin, with the experimental animals being euthanized at the Veterinary Medicine Department's Undergraduate Laboratory within the same university.

3.1 Materials

3.1.1 Chemicals and Reagents

All the substances utilized in this research were of analytical quality. The Aluminum oxide nanoparticles (Al_2O_3) were acquired from Sigma Aldrich, South Africa. Distilled water was obtained from Biochemistry department, unit of Benin City. Formaldehyde, formalin, normal Saline, vitamin C and chloroform were procured from Pyrex-ig scientific company, shop 14, Gabano plaza, opp UBTH gate, Ugbowo Lagos road, Benin City. Phosphate buffer was obtained from Quality Analytical Laboratory, Benin City.

3.1.2 Sample collection and preparation

3.1.3 Source of Nanoparticles used

The nanoparticle used in this study was purchased from Sigma Aldrich, South Africa.

3.1.4 Test Organism

The organism selected for this study is the male wistar rat. Thirty (30) individuals of these organisms were purchased from Animal house at the department of Anatomy, University of Benin City.

3.1.5 Apparatus/Equipment

Plastic Cages, ceramics plate, universal containers, 5ml syringe, plain containers, EDTA containers, plain tubes, biros, permanent markers, masking tapes, pasture pippettes, filter paper, microscopic slides, plastic petri dish and gavage were purchased from Pyrex-Ig Scientific Company, Shop 14, Gabano Plaza, Opp Ubth Gate, Ugbowo Lagos Road, Benin City. Table top centrifuge was purchased from Harris (England), ceramic mortar and pestle were obtained from Medax (England). Burettes, beakers, (50, 100, 250 and 500 mL), conical flasks, cuvettes, heparinized sample bottles, incubator, micropipettes, refrigerator, round bottom-flask, spatula, stirrer, test tubes and racks, water distiller, Sensitive weighing balance (Methler, H8) were products of Pyrex (England).

3.2 Methods

3.2.1 Animal experimentation procedure

Thirty male rats of the Wister strain, weighing between 80 and 120 grams and deemed healthy, were acquired from the animal facility at the Department of Anatomy, University of Benin, located in Edo State, Nigeria, to be utilized in the study. The rats were acclimatized for a period of one (1) week. The rats were accommodated in plastic cages, with six animals per cage, and these cages were situated within a well-ventilated rat house. They were given rat feed and clean water for drinking, during the period of acclimatization.

3.2.2 Toxicity test

After the period of acclimatization, the nanoparticle was administered to the rats for seven (7) days. The nanoparticles were administered to the rats via intraperitoneal injection ("Intraperitoneal" refers to a method of administering substances directly into the peritoneal cavity, which is the space within the abdomen that surrounds the abdominal organs). And the rats were given water via gavage (“gavage” refers to the forced delivery of food or medications, particularly to an animal, usually via a tube running down the throat to the stomach).

Thirty male rats of the Wistar strain were divided into five groups. These groups included a control group receiving distilled water, and four (4) treatment groups receiving doses of aluminum oxide nanoparticles at 0.05, 0.08, 0.12, and 0.2ml, respectively. They were observed on a daily basis during the period of administration.

3.2.3 Grouping and treatment of experimental animals (Experimental design)

Procedure of stock solution:

To dissolve the nanoparticles (aluminum oxide), add 1.0g of Al_2O_3 to 10ml of distilled water, water in a plan tube, shake vigorously to allow proper mixing.

The volume administered to each rat was calculated using the formular:

i. Convert initial weight from mg – g
ii. Convert kg – g
iii. Convert g – ml (1g – 10ml)

Volume = dose × weight/ Conc. of stock

Concentration of Nanoparticles Administered to Rats:

Control group: 1 ml of Distilled water

Group 1: 0.05 ml of aluminum oxide nanoparticles

Group 2: 0.08 ml of aluminum oxide nanoparticles

Group 3: 0.12 ml of aluminum oxide nanoparticles

Group 4: 0.2 ml of aluminum oxide nanoparticles

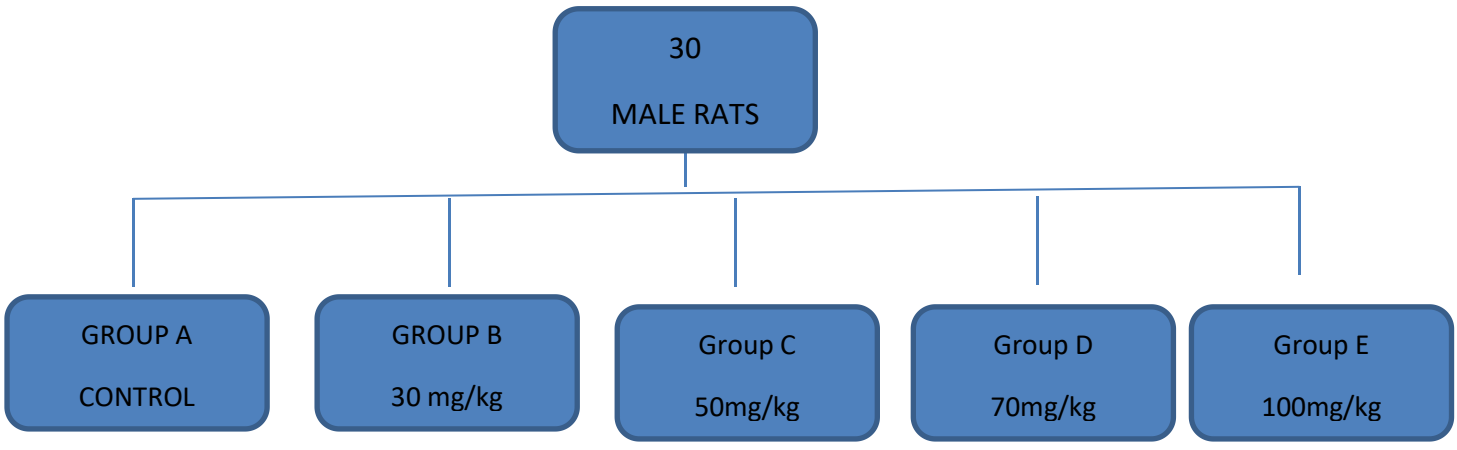

Fig 3.2 schematic representation of the experimental design

Plate 3.1 Preparing grouped experimental animals for treatment and acclimatization

Plate 3.2 Intraperitoneal administration dissolved in distilled water to the experimental animals

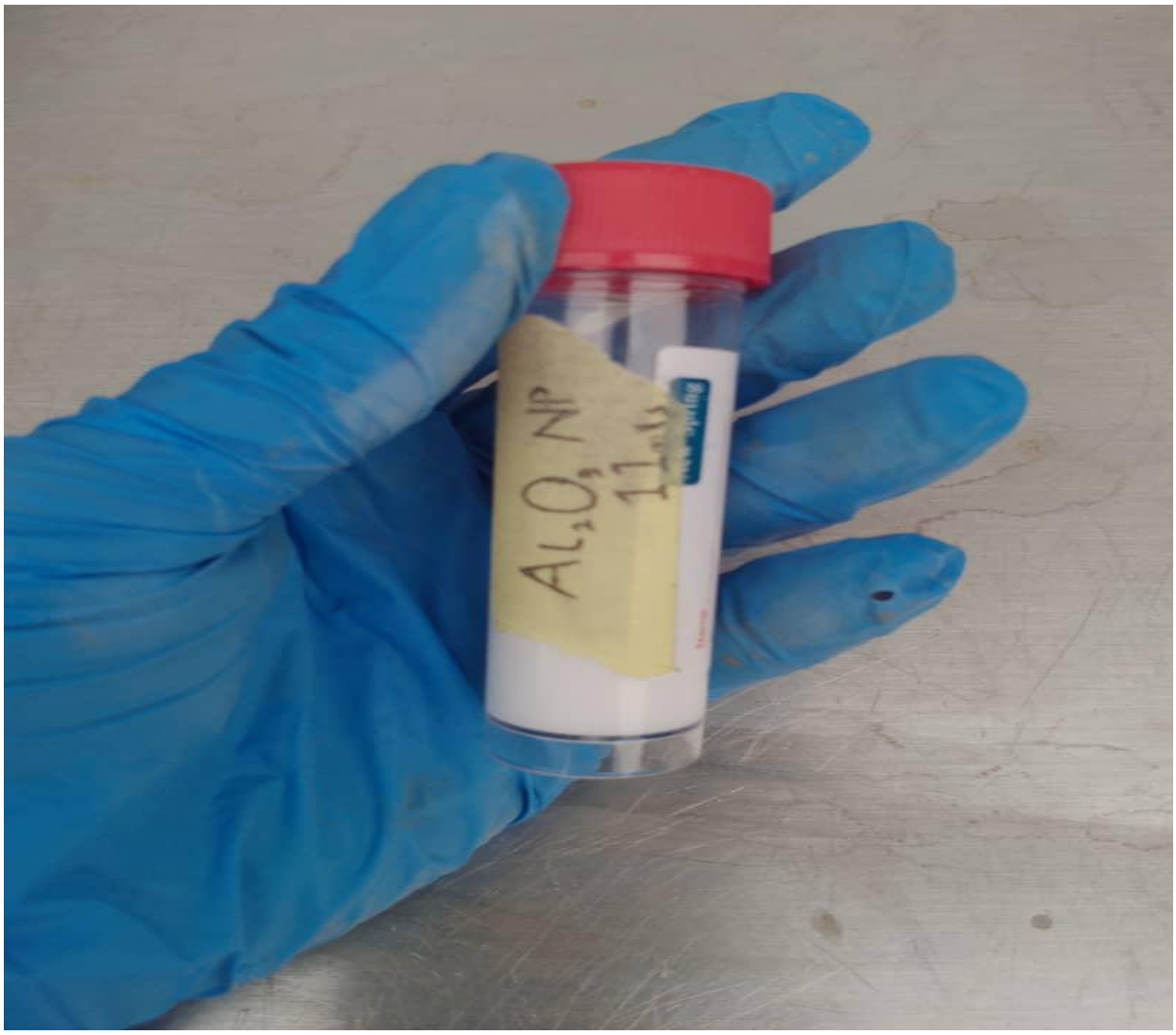

Plate 3.3 Al_2O3 nanoparticles stock solution

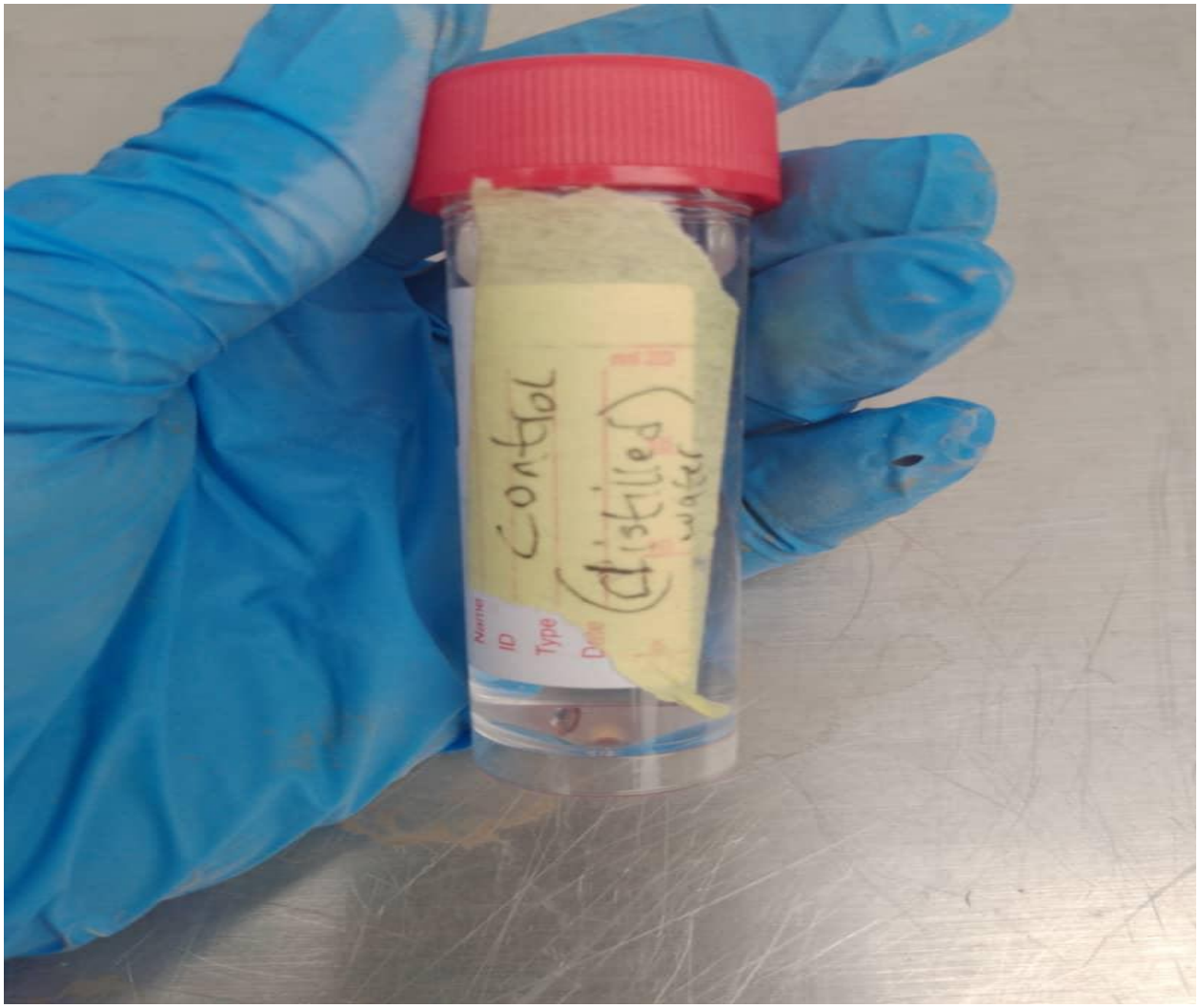

Plate 3.4 Distilled water for control group

3.2.4 Sacrifice of experimental animals

The rats in the experiment were subjected to chloroform exposure. Chloroform is a volatile organic compound, meaning it can easily turn into a gas and be inhaled, or it can be absorbed through the skin. This suggests that the rats were likely exposed to chloroform either by breathing it in or through direct contact with their skin during the experiment. Following their exposure to chloroform, the rats showed signs of weakness. This weakness is likely caused by the suppressive impact of chloroform on their nervous system and overall well-being.

The rats were sacrificed by gastrointestinal dissection. The testis and epididymis organs were removed using dissecting scissors and forceps, and then placed in a refrigerator at 4°C for storage. Samples taken from each organ were preserved in formalin and prepared for histopathological analysis. The testis and epididymis underwent cleaning and rinsing using a solution of 1.15% normal saline, after cleaning, they were dried using filter paper, weighed, and subsequently chopped into small pieces and homogenized using a mortar and pestle. Following homogenization, the samples were centrifuged at 10,000 g for 10 minutes in a centrifuge, and the resulting supernatant was collected for biochemical analysis.

Additionally, the testes and epididymis were macerated to extract mature sperm, which were then analyzed on a slide with normal saline to assess their motility, including the type of movement exhibited.

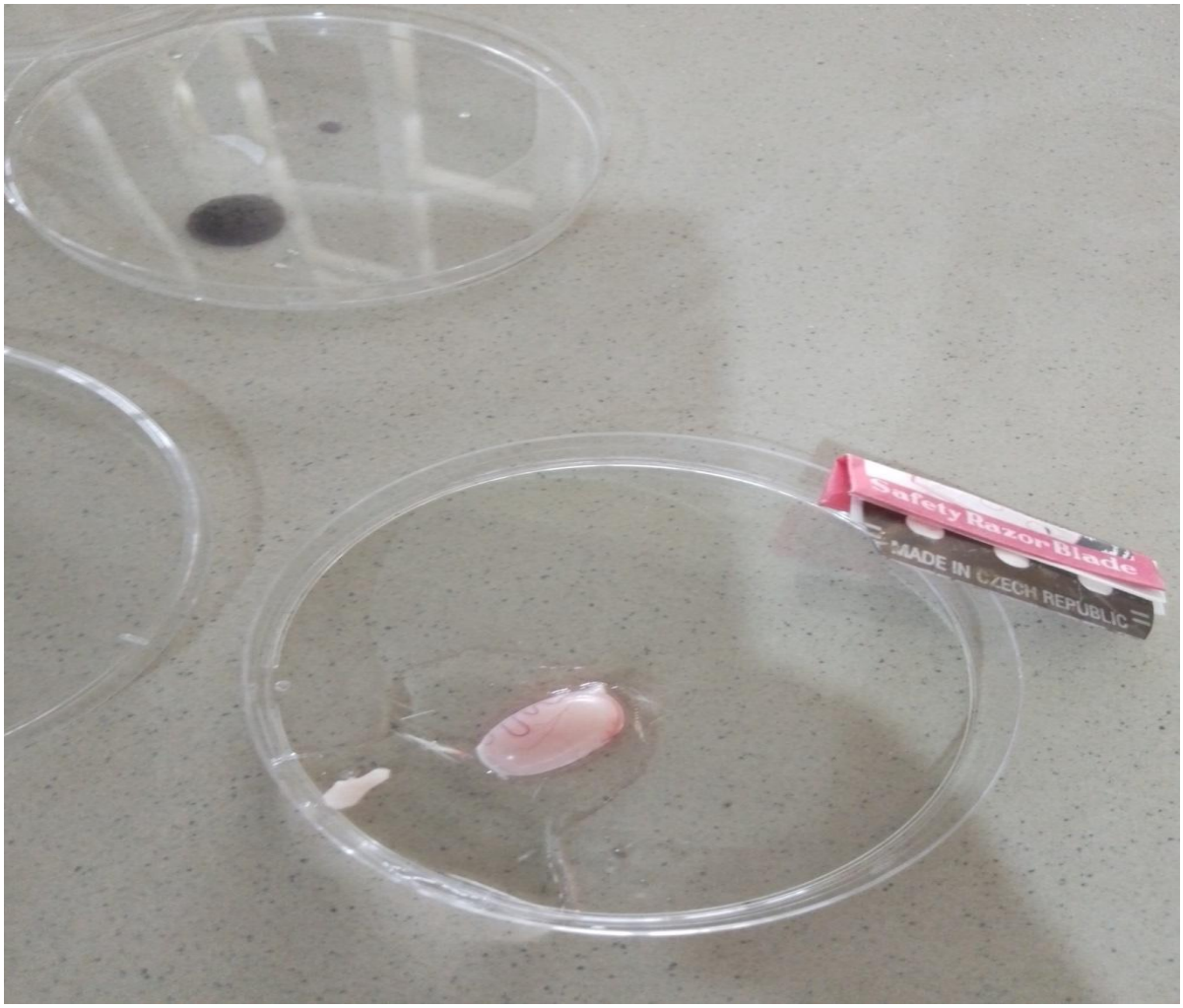

Plate 3.5 Epididymis and Testis placed in normal saline on a plastic dish, getting ready for maceration to conduct sperm analysis

Plate 3.6 Macerated epididymis placed in normal saline on a microscope slide, prepared for observation

Plate 3.7 Observing sperm cells motility under a light microscope

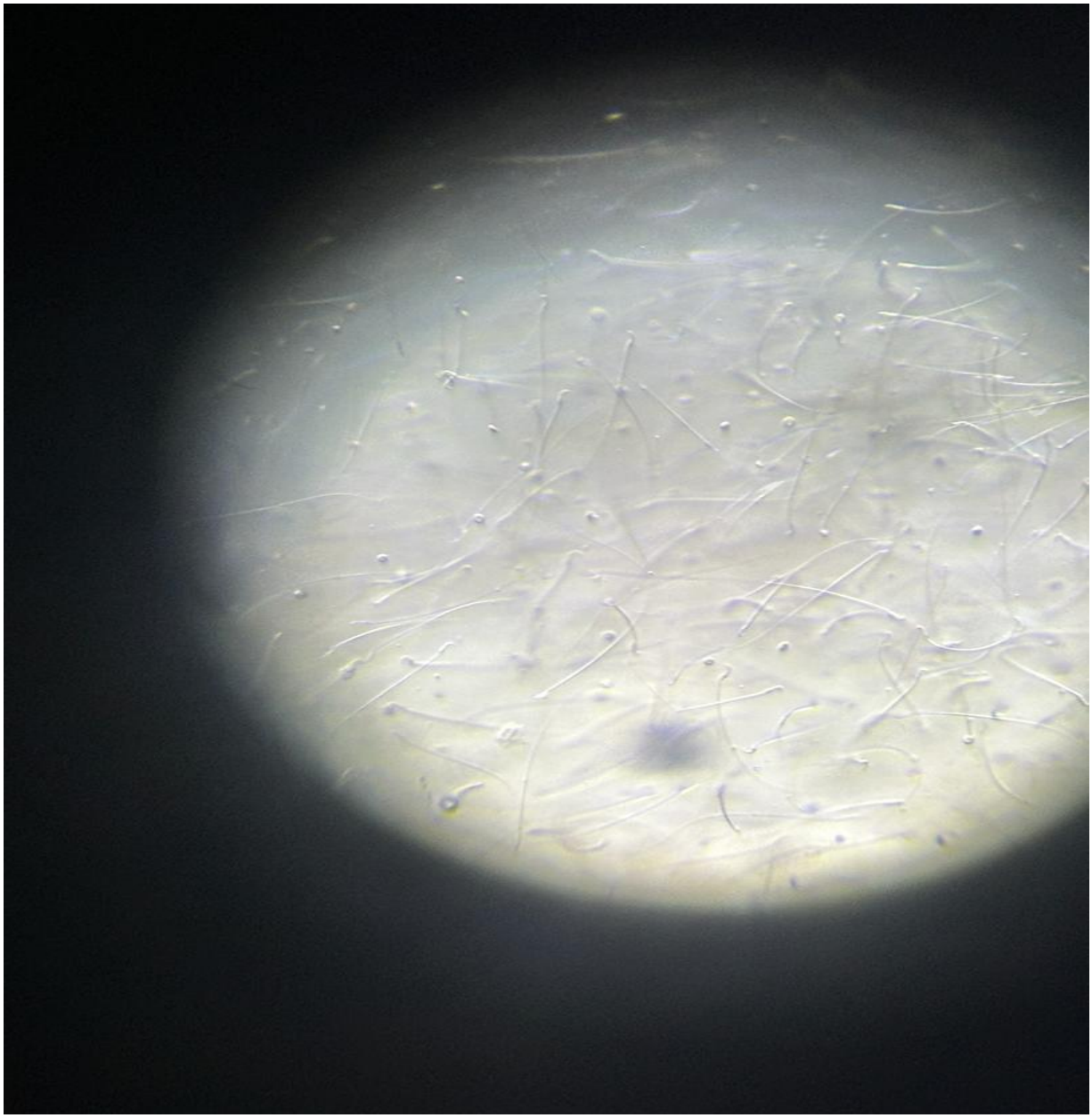

Plate 3.8 Sperm cells viewed under a light microscope at 10X magnification

3.3 Clinical biochemistry parameters

3.3.1 Hormonal assays

3.3.2 Laboratory method for assaying Luteinizing Hormone (LH)

Luteinizing hormone levels were measured using an immunoenzymometric assay (Type 3) following the procedure outlined by Kosasa (1981).

3.3.3 Test Procedure

Each serum reference, control, and specimen to be examined were duplicated and formatted in the microplate's wells, with each assigned well containing 0.01 ml (10 µl) of the appropriate serum reference, control, or specimen. Subsequently, 0.05 ml (50 µl) of the working LH enzyme reagent was added to all assigned wells, followed by gentle swirling for proper mixing for 20-30 seconds. After covering the microplate, it was allowed to incubate for 60 minutes at room temperature, then the microplate contents were discarded by aspiration and the plate blotted dry. Following this, 0.35 ml (350 µl) of wash buffer was added and aspirated from each well, repeated twice. Subsequently, 0.100 ml (100 µl) of working substrate solution was added to all wells and incubated for 15 minutes at room temperature. Finally, 0.050 ml (50 µl) of stop solution was added to each well, gently mixed for 15-20 seconds, and the absorbance was read at 450 nm using a microplate reader, with a reference wavelength of 620-630 nm to minimize well imperfections (Danzer and Braunstein, 1980; Kosasa, 1981)

3.3.4 Laboratory method for assaying Follicle Stimulating Hormone (FSH)

Follicle stimulating hormone levels were measured using an immunoenzymometric assay (Type 3) as outlined by Odell and Parlow (1981).

3.3.5 Test Procedure:

Each well of the microplate, including those for serum reference, control, and specimen, was duplicated and prepared accordingly. The designated wells received 0.050 ml (50 µl) of the appropriate serum reference, control, or specimen, followed by the addition of 0.100 ml (100 µl) of the working FSH enzyme reagent to all wells. After gentle swirling for 20-30 seconds, the microplate was covered and allowed to incubate for 60 minutes at room temperature. Following this, the contents of the microplate were discarded, and the plate was blotted dry. Subsequently, 0.350 ml (350 µl) of wash buffer was added to each well and aspirated, repeated twice. Next, 0.100 ml (100 µl) of working substrate solution was added to all wells, followed by incubation for 15 minutes at room temperature. Finally, 0.050 ml (50 µl) of stop solution was gently mixed into each well for 15-20 seconds. Absorbance readings were then taken at 450 nm using a microplate reader, with a reference wavelength of 620-630 nm to minimize well imperfections (Odell and Parlow, 1981).

3.3.6 Laboratory method for assaying prolactin (PRL)

The determination of prolactin hormone was conducted using an immunoenzymometric assay (Type 3) following the procedure outlined by Signorella and Hymer (1984).

3.3.7 Test Procedure:

Each well of the microplate, including those designated for serum reference, control, and specimen, was duplicated and prepared accordingly. 0.025 ml (25 µl) of the appropriate serum reference, control, or specimen was added to the assigned wells, followed by the addition of 0.100 ml (100 µl) of the working PRL enzyme reagent to all wells. After gently swirling and mixing for 20-30 seconds, the microplate was covered and allowed to incubate at room temperature for 60 minutes. Subsequently, the contents of the microplate were discarded, and the plate was blotted dry. 0.350 ml (350 µl) of wash buffer was added to each well and aspirated, repeated twice. Following this, 0.100 ml (100 µl) of working substrate solution was added to all wells and incubated for 15 minutes at room temperature. Lastly,

0.050 ml (50 µl) of stop solution was gently mixed into each well for 15-20 seconds. Absorbance readings were then taken at 450 nm using a microplate reader, with a reference wavelength of 620-630 nm to minimize well imperfections.

3.3.8 Laboratory method for assaying Progesterone

The determination of progesterone hormone was carried out using a competitive enzyme immunoassay (Type 7) following the procedure outlined by Ratcliffe (1982).

3.3.9 Test Procedure:

Each well of the microplate, including those designated for serum reference, control, and specimen, was duplicated and prepared accordingly. 0.025 ml (25 µl) of the appropriate serum reference, control, or specimen was added to the assigned wells, followed by the addition of 0.050 ml (50 µl) of the progesterone biotin reagent to all wells. After gently swirling and mixing for 20-30 seconds, the microplate was covered and allowed to incubate at room temperature for 60 minutes. Subsequently, the contents of the microplate were discarded, and the plate was blotted dry. 0.350 ml (350 µl) of wash buffer was added to each well and aspirated, repeated twice. Following this, 0.100 ml (100 µl) of working substrate solution was added to all wells and incubated for 15 minutes at room temperature. Lastly, 0.050 ml (50 µl) of stop solution was gently mixed into each well for 15-20 seconds. Absorbance readings were then taken at 450 nm using a microplate reader, with a reference wavelength of 620-630 nm to minimize well imperfections.

3.3.10 Laboratory method for assaying Estradiol (E2)

The measurement of estradiol hormone was conducted using a delayed competitive enzyme immunoassay (Type 9) following the procedure outlined by Abraham (1969).

3.3.11Test Procedure

Each well of the microplate, including those designated for serum reference, control, and specimen, was duplicated and prepared accordingly. 0.025 ml (25 µl) of the appropriate serum reference, control, or specimen was added to the assigned wells, followed by the addition of 0.050 ml (50 µl) of the estradiol biotin reagent to all wells. After gently swirling and mixing for 20-30 seconds, the microplate was covered and incubated at room temperature for 60 minutes. Subsequently, the contents of the microplate were discarded, and the plate was blotted dry. 0.350 ml (350 µl) of wash buffer was added to each well and aspirated, repeated twice. Following this, 0.100 ml (100 µl) of working substrate solution was added to all wells and incubated for 15 minutes at room temperature. Lastly, 0.050 ml (50 µl) of stop solution was gently mixed into each well for 15-20 seconds. Absorbance readings were then taken at 450 nm using a microplate reader, with a reference wavelength of 620-630 nm to minimize well imperfections.

3.3.12 Laboratory method for assaying Testosterone

The concentration of testosterone was assessed using a competitive enzyme immunoassay (Type 7) following the procedure outlined by Ismail (1986).

3.3.13 Test Procedure:

Each well of the microplate, including those designated for serum reference, control, and specimen, was duplicated and prepared accordingly. 0.010 ml (10 µl) of the appropriate serum reference, control, or specimen was added to the assigned wells, followed by the addition of 0.050 ml (50 µl) of the working testosterone enzyme reagent to all wells. After gently swirling and mixing for 20-30 seconds, the microplate was covered and allowed to incubate at room temperature for 60 minutes. Subsequently, the contents of the microplate

were discarded, and the plate was blotted dry. 0.350 ml (350 µl) of wash buffer was added to each well and aspirated, repeated twice. Following this, 0.100 ml (100 µl) of working substrate solution was added to all wells and incubated for 15 minutes at room temperature. Lastly, 0.050 ml (50 µl) of stop solution was gently mixed into each well for 15-20 seconds. Absorbance readings were then taken at 450 nm using a microplate reader, with a reference wavelength of 620-630 nm to minimize well imperfections.

3.4.3 Data Analysis

Statistical and graphic analyses were done using GraphPad Prism 5 software. A grouped table option was used to enter replicate values of the data, in side-by-side columns. Data in endpoint and chronological methods were presented as mean ± SEM (standard error of mean), and the level of significance was set at $p < 0.05$ (95% confidence intervals). Comparison between control and treatment was carried out using one-way ANOVA, and Dunnett's multiple comparison post-HOC test.

3.5 OXIDATIVE STRESS

3.5.1 Determination of Catalase (Cat)

Catalase (CAT) activity was estimated by the method described by Cohen *et al.,* (1970).

3.5.1.1 Reagents

Hydrogen peroxidase (H_2O_2)

Suphuric acid (6M) H_2SO_4

Preparation of reagents

0.01M $KMnO_4$ was prepared by distilling 0.158g of $KMnO_4$ in 100ml of distilled water. Phosphate buffer (pH 7.4) 0.426g of $NaHPO_4$ and 0.240g of NaH_2PO_4 was weighed and

dissolved in 100ml of distilled water. 6M H_2SO_4 and 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.

3.5.1.2 Procedure

To an unknown volume of plasma (0.5ml), 5.0ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30min. The reaction was stopped by adding 1.5ml of 6M H_2SO_4 and 7ml of 0.01M $KMnO_4$. These were mixed by inversion and allowed to stand for 10min. The absorbance was read at 480nm within 30-60 seconds against distilled water. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as µmoles of H_2O_2 decomposed/min/mg/protein.

Calculation

$$\text{Activity} = \frac{OD/min \times V}{M \times V \times L \times Y}$$

Where OD = Absorbance

L= Light path

V= Total volume of reaction sample

M= Molar coefficient of H_2O_2 (40/m/cm)

V= Volume of sample

Y= mg protein in the sample

3.5.2 Estimation of Superoxide Dismutase Actvity (SOD)

This was determined according to teh methods of Masra and Fridorich (1972)

3.5.2.1 Principle

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

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

3.5.2.2 Reagent and preparation

Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na2CO3, 0.2604g NaHCO3 and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide.

Hydrochloric acid (0.005M): This was prepared by adding 0.044 concentration of HCL to 99.96mls of distilled water.

Adrenaline solution (0.3mM): This was prepared by dissolving 0.01098g of adrenaline in 100mls of 0.005M HCL solution.

Plasma volume of 100ml was mixed with 125ml of carbonate buffer and 150ml of adrenaline solution. 100ml of distilled water was mixed with 1.25ml of carbonate buffer as reference sample. These were mixed and absorbance read at 420nm.

These were mixed and read at 420nm

%inhibition= (O.D test - ODref) x 100

OD test

Enzyme concentration can thus be calculated

unit/mg protein = % inhibition

50 x Y

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

3.5.3 Estimation of Gluthathione Peroxidase (Gpx)

This was determined according to Nyman (1959)

3.5.3.1 Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown color disposition, read at 420nm.

3.5.3.2 Reagent and preparation

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

3.5.3.3 Procedures

To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H2O2, 1.5ml of distilled water and 2.5ml of pyrogallol was added.

The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Calculations

Activity= OD/min x vt x Df

E x Vs x Y

OD= Absorbance of test

Vt= Total volume of reaction mixture

Df= Diution factor = 1

E= Molar extinction co-efficient (12/m/cm)

Vs= Volume of sample

Y= mg of protein used

3.5.4 Determination of Malondialdehyde (Mda)

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

3.5.4.1 Principle

Malonaldehyde which is a product of lipid peroxidation react with thiobabituric acid (TBA) to give a red species.

3.5.4.2 Procedure

A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuged at 1000g for 10min. The absorbance was determined using the formula;

MDA (mol/mg protein) = A x V x 100

M x V x Y

A= Absorbance

V= Total volume of reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

CHAPTER FOUR

4.0 RESULTS

Figure 4.1 Change in body weight of experimental group.

CHAPTER FIVE

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