INVESTIGATING THE EFFECTS OF *ginseng* ON CADMIUM-INDUCED TESTICULAR TOXICITY IN WISTAR ALBINO RATS

BY

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BMS2005027

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

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UNIVERSITY OF BENIN, NIGERIA

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BENIN CITY.

THIS PROJECT IS SUBMITTED TO:

THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

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SUPERVISOR:

DR. NOSA TERRY OMORODION

OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by EHIOZEE EDNA ISOKEN (MISS) with matriculation number: BMS2005027 in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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PROF. VICTOR .O. EKUNDINA. DATE

(External Supervisor)

DEDICATION

I dedicate this project work to God Almighty, for making this work a great success, to my lovely parent MR AND MRS EHIOZEE and family member for their constant support throughout the process.

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I give thanks to God Almighty for His grace upon my life and for seeing me through this project work.

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ABSTRACT

Cadmium (Cd), a pervasive environmental toxicant, has been widely implicated in male reproductive dysfunction due to its ability to induce oxidative stress, disrupt endocrine signaling, and impair testicular architecture. In recent years, herbal medicine have thrived in therapeutic usage, ginseng has been known to be a potent Korean herb.The protective role of ginseng is attributed to its antioxidant, anti-apoptotic, and anti-inflammatory properties, which counteract cadmium-induced oxidative stress and cellular damage. This study aimed to investigate the histopathological and functional effects of *ginseng* on cadmium-induced testicular toxicity in Wistar albino rats. Animal model was used for this study, twenty male rats were used and randomly divided into four groups: Group A (control), Group B (Cd-exposed), Group C (Cd + 200 mg/kg ginseng), and Group D (Cd + 400 mg/kg ginseng). Cadmium chloride was administered imtraperitoneally at 1 mg/kg, while ginseng was administered orally, all administration was for three weeks after 14 days acclimatization. Parameters assessed included body and testicular weight, sperm characteristics (count, motility, morphology), and histopathological changes in testicular tissue. Results demonstrated a statistically significant reduction in testicular weight, sperm count, and progressive motility in cadmium-exposed rats compared to controls ($p < 0.05$). Histological examination revealed degeneration and shrickening of seminiferous tubules, germ cell loss in Cd-treated groups. Co-administration of *ginseng*, particularly at 400 mg/kg, significantly ameliorated these effects, as evidenced by improved sperm parameters, restoration of testicular architecture. Putting together all data from this study, this study provides evidence that *ginseng* exerts a dose-dependent protective effect against cadmium-induced testicular toxicity in Wistar rats. These findings highlight its therapeutic potential as a natural adjunct in mitigating heavy metal-associated reproductive dysfunction. Further study into optimal dosage for therapeutic usage is of great importance.

CHAPTER ONE

INTRODUCTION

1.1 **BACKGROUND OF STUDY**

Cadmium (Cd), alongside arsenic, lead, mercury, and chromium, is a heavy metal that does not have a physiological function and is often considered a toxicant. Many different forms of exposure to cadmium have been shown over the past century, with cadmium being present in the environment as a result of many human activities (Giuseppe *et al*.,2020). Due to its versatility, it is used in a variety of industrial operations, including the generation of Cd batteries, color pigments, corrosion inhibitors, and neutron generators in nuclear power plants (Godt *et al*., 2006). Human exposure to cadmium can have major health effects, especially for workers who are exposed at work (Alli, 2019). Because of this heavy metal's toxicity, both people and animals are at serious danger for health problems. It can enter the body through food, water, or the air and is widely disseminated in the environment. Over time, cadmium accretion in the different visceral organs (liver, lungs, kidney, and testis) is said to impair the function of these organs. Recently studies have revealed that the testes are highly sensitive to cadmium (Waseem *et al*., 2022). Many investigations have revealed that mammalian testes are very sensitive against cadmium, which causes toxicity in male reproductive organs, particularly the testicles and sperm parameters, because of their active cell division and metabolism (Wang *et al*., 2022). Studies aimed at discovering naturally occurring food ingredients that can be safely and efficiently employed to reduce heavy metal toxicity are greatly appreciated. Through dietary intervention, a number of plants, including garlic, ginger, onions, kola nuts, and green tea, have been utilized to treat lead and cadmium toxicity (Zhai *et al*., 2015; Osemwegie *et al*., 2017).

ginseng is a perennial herb of the family *Araliaceae*. For millennia, *P. ginseng* has been traditionally used as a medicine in Asia, particularly in Korea, China and Japan. More recently, *ginseng* has become popular globally (Cho, 2012). Its roots have been traditionally used to

revitalize the body and mind, increase physical strength, prevent aging and increase vigor (Choi, 2008). It's roots (mostly), stems, leaves , and extracts have been used for maintaining immune homeostasis and enhancing resistance to illness or microbial attacks through effects on immune system (Kang and Min, 2012)., its medicinal constituents include ginsenosides, polyphenolic, acidic polysaccharides, and polyacetylenes compounds, with ginsenosides being the most pharmacologically active (Mashwa *et al*., 2025).It has demonstrated potent antioxidant, anti-inflammatory, and anti-apoptotic properties. Ginsenosides or ginseng saponins are the principal active ingredients in *ginseng* and more than thirty different ginsenosides have been identified (Mishra and Verma, 2017). Recent studies on the effects of ginsenosides on various diseases, particularly on diabetes, hypertension and cancer. Ginseng improves glucose homeostasis and insulin sensitivity. It exerts cytotoxic and anti-metastatic activities against various kinds of cancer cell lines, and induces differentiation or apoptosis of several cancer cells (Park *et al*., 2005). Its potential to counteract toxicological damage in reproductive organs has been observed in various experimental studies. This study aimed to evaluate the potential of *ginseng* in attenuating histopathological changes in the testes of Wistar rats exposed to cadmium, thereby contributing to potential therapeutic strategies for environmental toxicant-induced infertility.

1.2 STATEMENT OF PROBLEM

Heavy metals present serious risks to human health and the environment, especially in light of human activities like mining, industrialization, and the use of phosphate fertilizers. Cadmium (Cd) is a metal that is particularly problematic for people who are exposed to it at work because of its extensive environmental distribution and serious health risks (Genchi *et al*., 2020; Smereczański and Brzóska, 2023). It poses a health risk for both humans and animals. It is naturally occurring in the environment as a pollutant that is derived from agricultural and

industrial sources. Because cadmium builds up in many body organs and can have harmful effects on health, chronic exposure to cadmium is especially concerning (Bernard, 2008). Cadmium exposure as an environmental pollutant poses significant risks to male reproductive health by inducing testicular toxicity through oxidative stress, apoptosis, and inflammation (Nna *et al*., 2017), thereby reducing sperm count, impairs sperm motility, and causes structural damage to testicular tissue, making the male reproductive system particularly vulnerable (Ilieva *et al*., 2020). This toxic effect emphasizes how urgently we need efficient treatments to lessen the harmful effects of cadmium poisoning. Due to the health risk cadmium poses to man, different treatment option have been explored. While synthetic antioxidants like melatonin have demonstrated protective effects (Bakarat *et al*., 2025), natural alternatives such as *Panax ginseng* show promise due to their antioxidant and anti-apoptotic properties (Shojaeepour *et al*., 2021). Existing studies confirm that *ginseng* ameliorates cadmium-induced sperm quality decline and germ cell apoptosis in Wistar rats, and synergistic effects with melatonin have been documented. However, critical gaps remain. Studies like (Shojaeepour *et al*., 2021) use fixed ginseng doses (e.g., 100 mg/kg), leaving optimal dosing regimens and long-term safety profiles uncharacterized. While Bakarat *et al*, (2025), highlights melatonin's superiority in reducing autophagy, direct comparisons between *ginseng* and other antioxidants in mitigating cadmium-specific testicular damage are limited. Most evidence centers on biochemical markers, with insufficient data on *ginseng's* ability to restore testicular histoarchitecture and spermatogenic cell viability post-cadmium exposure. This study addresses these gaps by systematically evaluating *Panax ginseng*'s efficacy in alleviating cadmium-induced testicular dysfunction in Wistar albino rats, elucidating its mechanisms via anti-oxidative, anti-apoptotic, and anti-inflammatory biomarkers, and establishing dose-dependent therapeutic thresholds.

1.3 STUDY JUSTIFICATION

Heavy metals poses a significant health danger and concern, amongst the numerous heavy metals, cadmium (Cd) is of uttermost concern as its exposure to industrial workers calls for quick fixes to lessen their detrimental effects on reproductive health. Cadmium (Cd) is a heavy metal and a major environmental toxicant. The general population is exposed to Cd via contaminants found in drinking water and food (WHO, 2000; ATSDR, 2008), while occupational exposure to Cd usually takes place during mining or manufacturing of batteries and pigments that utilize Cd. Industrial activities, such as smelting and refining of metals, and municipal waste incineration also release Cd to the atmosphere as cadmium oxide, chloride or sulfide (Erica *et al*., 2010). Recent studies have illustrated that the testis is highly sensitive to cadmium toxicity. Its toxic effect are primarily mediated through oxidative stress, inflammation, apoptosis, and disruption of hormonal balance in the testes (Erica *et al*., 2010). Given the widespread cadmium exposure finding protective agents is critical, which therefore require a strong need to investigate and validate efficient remediation techniques because of the great health risks connected with long-term exposure to cadmium, its broad distribution in the environment, and its versatility and extensive use in industrial processes. A promising approach to solving this problem is through the use of natural and traditional remedies. Numerous plants have been investigated for their ability to mitigate the effects of heavy metal toxicity, which has led to the investigation of dietary interventions as a potential course of action. Exploring *panax ginseng* protective abilities may offer a natural, accessible therapeutic strategy to mitigate male reproductive toxicity caused by cadmium, which currently lacks clinical interventions (Park *et al.*, 2013). *Panax ginseng* is notable because of its well-established anti-inflammatory, antioxidant, and protective qualities. Previous research has shown the plant's beneficial effects in reducing reproductive toxicity and other health

conditions. Investigating *Panax ginseng* abilities in mitigating cadmium testicular toxicity is a crucial step towards validating a natural, effective remedy for cadmium toxicity, addressing a significant environmental health challenge, and providing a foundation for future research and practical applications in the field. This study may open the door to novel dietary guidelines and treatments, which would ultimately benefit the general public's health and the quality of life for those who are exposed to heavy metals.

1.4 SIGNIFICANCE OF STUDY

Infertility constitutes a significant global health concern. Cadmium (Cd), a hazardous heavy metal, exerts deleterious effects on the reproductive system (Barakat *et al*., 2025). This has lead to and have added to the increasing rate of infertility especially among workers who are at higher risk due to continuous exposure from industrial materials. Considering the testicular damage, impaired spermatogenesis, reduced sperm quality and spoerm count caused to exposure to cadmium, it is essential to devise novel pharmaceutical strategies to mitigate this adverse effect (Barakat *et al*., 2025). The search for effective natural intervention to counteract these effect is a critical area of reproductive toxicology and public health. For multiple important reasons, the research on the protective effects of Panax ginseng against cadmium-induced reproductive toxicity is extremely important in the following ways.

Firstly, this research shows significant impact on public health. Determining effective interventions is critical because rising levels of cadmium contamination pose serious risks, particularly to male reproductive health (Zhao *et al*., 2023; Mognetti *et al*., 2024). Testicular damage, decreased sperm count, and decreased sperm motility can all result from cadmium exposure (Ige *et al*., 2012). Secondly *Panax ginseng* is a medicinal plant widely recognized for

its antioxidant and anti-apoptotic capabilities and has shown clinical efficacy in male reproductive health (Barakat *et al*., 2025). The findings of this research support that *panax ginseng* is a potential therapeutic agent for protecting male reproductive health especially in populations at risk of environmental or occupational cadmium exposure (Shojaeepour *et al*., 2021). This study has implication for fertility preservation and the management of male infertility linked to environmental toxins. Thirdly, this research highlight the mechanism by which panax ginseng exerts it's protective effect thereby improving our knowledge of the mechanisms underlying the actions of this medicinal plant as well as giving insight to natural antioxidants and protective agents present in medicinal plants. This opens new avenues for natural medicine and environmental toxicology studies. Fourth, this study shows interest in occupational and environmental health. There is a significant risk of cadmium exposure for industrial workers in sectors like metal processing, welding, and battery manufacturing (Bonberg *et al*., 2017). The results of this study may influence the use of dietary supplements or interventions as preventive measures for occupational groups that are more vulnerable to workplace health and safety hazards. To crown it all, this study supports and encourage African traditional medicine practices that have been employed for a range of health advantages. Acknowledging the effectiveness of these conventional treatments can encourage the fusion of traditional wisdom with contemporary scientific findings in the advancement of public health and encouraging cultural heritage preservation.

AIMS AND OBJECTIVES

Aim: To investigate the histopathological effects of *Panax ginseng* on cadmium-induced toxicity in the testis of albino rats.

Objectives:

1. To evaluate the histopathological changes in the testicular tissue of albino rats exposed to cadmium.
2. To assess the protective effects of *Panax ginseng* on cadmium-induced testicular damage.
3. To compare the reproductive parameters (sperm count, motility, morphology) between treated and control groups.

RESEARCH QUESTION

1. What histopathological changes occur in the testicular tissue of albino rats exposed to cadmium?
2. Does *Panax ginseng* provide histopathological protection against cadmium-induced testicular damage?
3. How do reproductive parameters (sperm count, motility, morphology) differ between cadmium-exposed rats treated with *Panax ginseng* and untreated controls?

HYPOTHESIS

Alternative Hypothesis (H1): *Panax ginseng* serves as a reliable treatment alternative for cadmium induced testicular toxicity

CHAPTER TWO

LITERATURE REVIEW

2.1 REVIEW ON *Panax ginseng*

Panax ginseng is a perennial herb native to East Asia primarily cultivated in Korea, China, Russia, and Japan. The word "panax" comes from Greek, meaning "all-healing" or "panacea" (Kim, 2012), the specie name 'Ginseng' comes from the Chinese word "rensheng" which means "human", it is named after its root shape, which is well branched and resembles the human body (Ikeuchi *et al*., 2022). It holds significant therapeutic value in traditional medicine practices and is a widely used natural product around the world (Zhou *et al*., 2024). While these plants have been used as important herbal medicines, especially in eastern Asia, they are also used in foods, drinks, dietary supplements, and other daily uses. Although *ginseng* usually needs more than four years to be harvested and used, a large amount of *ginseng* is grown and distributed (Ikeuchi *et al*., 2022). In the modern era, *ginseng* attracts great interest because of its various pharmacological and therapeutic effects on aging, cancer, the cardiovascular system, diabetes, immune-regulatory function, and inflammation (Dong, 2020). It also be divided into the following categories based on the processing method: fresh *ginseng* (under 4 years old, freshly consumed), white *ginseng* (between four and 6 years old, prepared by peeling and oven- or air-dried), sun ginseng

(produced by steaming white ginseng under high temperatures and pressure), and red *ginseng* (6 years old, steamed without peeling), (Li *et al*., 2023). *ginseng* has been employed as a tonic to enhance bodily vigor and as an adaptogen to alleviate both mental and physical stress, fatigue and improve overall vitality (Patel and Rauf, 2017). Its root is the most commonly used part and has been a cornerstone of traditional Chinese and Korean medicine for centuries. Ginsenosides or *ginseng* saponins are the principal active ingredients in *ginseng* and more than thirty different ginsenosides have been identified (Mishra and Verma, 2017). Ginsenosides are unique to Panax species, many of which exist in minute amounts and are believed to be responsible for most of *ginseng's* actions (Mishra and Verma, 2017).

2.2 NUTRITONAL COMPOSITION AND NUTRITIONAL VALUE

ginseng has been widely used as dietary supplements and health substances in worldwide including East Asia (Korea, China, and Japan) due to its biological and pharmacological properties (anticancer, antioxidant, and anti-inflammatory activities) (Lee *et al*., 2021). It is valued primarily for its active compounds rather than as a significant source of macronutrients like protein, fat, or carbohydrates. The main active components of *ginseng* are commonly considered to be triterpene saponins, termed ginsenosides, more than 30 different kinds of ginsenosides have so far been isolated and their chemical structures elucidated (Kim, 2016), including widely studied neutralginsenosides (e.g., Rb1, Rc, Rb2, Rd, Rg1, and Re) and the less studied malonyl ginsenosides (e.g., M-Rb1, M-Rc, M-Rb2, and M-Rd) (Lee *et al*., 2020). The roots of *ginseng* may also contain important nutritional components including glucose, and fructose, sucrose, and maltose which are in bulk, and in addition, various amino acids are contained (Lee *et al*., 2015). Studies have reported that ginseng leaves are rich in polysaccharides, phenolics, flavonoids, and ginsenosides (Kim, 2016).

According to different aglycones, ginsenosides can be classified into three types: protopanaxadiol type, such as ginsenoside Rb1, Rc, Rb2, and Rd; protopanaxatriol type, such as ginsenoside Rg1 and Re; and oleanolic acid type, which includes ginsenoside Ro and polyacetyleneginsenoside Ro (Zu *et al*., 2018). However, non-saponin components have recently received a great attention for their antioxidant, anticancer, antidiabetic and immunomodulating activity.It contain 67 to 80 % carbohydrate, and 3 to 8 % ash on dry weight basis, 16 to 30 % dietary fiber, average energy value of 342 kcal (Jackson *et al*., 2024). *ginseng* seeds contain 15.0–26.6% (w/w, dry basis) oil, 17.9–22.1% (dry basis) crude lipids, 11.5–15.2% crude proteins (Kim *et al*., 2018). Other chemical constituents present in ginseng include polyacetylenes, phenol, essential oils, polysaccharides, microelements, and vitamins (Kim, 2016).

Table1: Root and pharmaceutically manufacturered pure *ginseng* (Pooja Paharia. 2024)

2.3 PHYTOCHEMICAL CONSTITUENTS AND MEDICINAL VALUE

Recent studies have reported the constituents of the roots of *ginseng*, as well as their leaves and stem, flower buds, and fruits. Based on these findings, the leaves and stem, flower buds, fruits, and roots have recently been used as functional food, cosmetics, and herbal medicines (Kim, 2012). The roots of these *ginsengs* have been used as herbal medicines in Asian countries, and their bioactive chemicals have been isolated. In 1854, Garriques performed the first chemical studies on *ginseng* (Garriques, 2016) and separated a saponin fraction from *P. quinquifolium*. Despite these findings, the components of ginseng were not studied again until 1963. Shibata *et al*, (1963), and Shibata *et al* (1965) isolated *ginseng* saponins from the root of *Panax ginseng* and identified their structures. These saponins were called ginsenosides. Since the report many researchers have isolated the components of Korean *ginseng*, American *ginseng*, and Notoginseng. Today, approximately 200 substances, such as ginsenosides, polysaccharides, polyacetylenes, peptides, and amino acids, have been isolated from *Panax ginseng* (Kim, 2012), According to the different structures, ginsenosides in *P. ginseng* are commonly divided into two types: damarmane-type tetracyclic triterpenoid saponins (including protopanaxadiol type, protopanaxatriol type, and C17 side-chain varied type), and oleanane type pentacyclic triterpenoid saponins (Geng *et al*., 2024).

2.3.1 saponin

Saponins are the major constituents isolated from the root of *ginseng* (Kim, 2012). Latest surveys show that saponins account for about 3–4 % of *Panax ginseng* (Park *et al*., 2015). Since Shibata *et al*. isolated prosapogenins in 1963, many kinds of saponins have been isolated from its roots, leaves and stem, flower buds, fruits, and seeds. Shibata *et al*. (1963) established the

chemical structures of main the prosapogenins 20Sprotopanaxadiol, 20S-protopanaxatriol, prosapgenin, and ginsenoside Rg_1, which were extracted from the dried root of *ginseng* (Kim, 2012). Kitagawa *et al* (1947) and Kitagawa *et al*. (1983) isolated malonyl ginsenosides Rb_1, Rb_2, Rc, and Rd from the dried root of ginseng. Subsequently, the ginsenosides Ro, Ra_1, Ra_2, Ra_3 Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, the notoginsenosides R4, 20-gluco-ginsenoside Rf, koryoginsenoside R1, and R2, and the ginsenosides Rb_1, Rb_2, Rc, and Rd, have been reported (Kim, 2012). In a study by Wang *et al* (2013), 28 ginsenoside were isolated including: Koryoginsenoside R1 (1), ginsenoside Rg1 (2), ginsenoside Rf (3), notoginsenoside R2 (4), ginsenoside Rg2 (5), notoginsenoside Fe (6), ginsenjilinol (7), ginsenoside Re5 (8), noto-ginsenoside N (9), notoginsenoside R1 (10), ginsenoside Re2 (11), ginsenoside Re1 (12), ginsenoside Re (13), ginsenoside Rs2 (14), ginsenoside Ro methyl ester (15), ginsenoside Rd (16), ginsenoside Re3 (17), ginsenoside Re4 (18), 20-gluco-ginsenoside Rf (19), ginsenoside Ro (20), ginsenoside Rc (21), quinquenoside-R1 (22), ginsenoside Ra2 (23), ginsenoside Rb1 (24), ginsenoside Ra1 (25), ginsenoside Ra3 (26), ginsenoside Rb2 (27), and notoginsenoside R4 (28). All isolated compounds are 20 (S) -protopanaxadiol or protopanaxatriol type triterpenoid saponins (Wang *et al*., 2013). The chemical components of the *Panax* species still draw wide interest in the last three years (2021−2023) to discover new active components he main ginsenosides isolated from *Panax* species (Mengxiang *et al*., 2024). They contain protopanaxadiol, protopanaxatriol, ocotillol, oleanolic acid, and C-17 side chain type (Yang *et al*., 2021).

2.3.2 polysaccharide

As an important pharmacodynamically active ingredient of *ginseng*, polysaccharides have received a high amount of attention (Liu *et al*., 2020). Ginseng polysaccharides (GPS) are mainly divided into two categories based on their monosaccharide composition, namely starch-

like glucans and pectin. Starch-like glucans include dextran and arabinogalactan (AG). Ginseng pectin is mainly composed of galactose (Gal), galacturonic acid (GalA), arabinose (Ara), and rhamnose (Rha) (Zhao *et al*., 2019).Two homogeneous *ginseng* polysaccharide fractions, GPII and GPIII, have been obtained, both fractions comprised glucose (Liu *et al*., 2020). Zhang *et al*, (2009) obtained the total sugar of *ginseng* through water extraction, and the main component was amyloid dextran, with a small amount of AG.

Pectin is a polysaccharide rich in GalA. Based on the ratio of Rha and GalA, pectin is composed of different domains, including rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Inngjerdingen *et al*., 2005). These types of pectin do not usually exist alone and are often composed of RG-I, homogalacturonan (HG), and AG structures (Liu *et al*., 2020). Sun *et al*, (2019) isolated a series of RG-I and RG-II domains from ginseng pectin by alkali saponification and endo-polygalacturonase hydrolysis. Gao et al. isolated and purified four kinds of acid pectins rich in RG-I and HG domains from ginseng, all of which contained AG side chains and a small amount of GalA. Zhang *et al* (2020) obtained four homogeneous, HG-rich pectin fractions by fractional purification. Pectins rich in RG-I domains often contain few or no HG domains, and side chains are composed of AG-I and AG-II, either alone or together. Pectins rich in HG domains often present different degrees of methyl esterification and acetylation, and the side chain often contains a small amount of AG or dextran. The total content of ginseng pectin Ara and Gal that is rich in AG domains exceeds 50 % and often contains few RG-I domains, which may exist as the core domain of the molecule. Wang *et al*, (2013) reported that the polysaccharide content of ginseng was 28.56 %.

2.3.3 Amino acids

Amino acids are important organic compounds containing carboxyl (-COOH) and amine (-NH_2) functional groups. On the basis of the number of acidic carboxylic groups and basic amino groups in the molecule, the amino acids are classified into three main groups: neutral amino acid (e.g., threonine, leucine, valine, tyrosine, proline, alanine, glycine, and serine), acidic amino acid (e.g., glutamic acid and aspartic acid), and basic amino acid (e.g., arginine, lysine, and histidine) (Liu *et al*., 2020). *Panax ginseng* contains at least 18 amino acids. Fourteen of these amino acids are highly accumulated, including arginine, glutamic acid, aspartic acid, glycine, leucine, alanine, proline, lysine, serine, threonine, proline, phenylalanine, isoleucine, and tyrosine (Liu *et al*., 2020). The amino acid content in Panax ginseng root is typically between 11% and 14% of dry weight (Liu *et al*., 2019). **Arginine** is consistently the most prevalent, accounting for as much as 67.5% of total free amino acids in some studies. Other amino acids such as **glutamic acid, leucine, and alanine** are also present in significant amounts (Lee *et al*., 2013).

2.3.4 polyacetylene

Polyacetylene is an organic compound in which a single bond and a triple bond alternate. “Polyyne” refers to the presence of several alkynes, the C_{17}-polyacetylenes, which include panaxynol (**1**) and its related epoxide panaxydol (**2**), have attracted remarkable interest mainly due to their biological activities (Knispe *et al*., 2013). Panaxynol was first isolated from roots of *P. ginseng* C.A. Meyer and described in 1964. To date, more than 16 polyacetylenes have been reported from *P. ginseng* (Knispe *et al*., 2013). C_{17} polyacetylenes have potential cytotoxic properties and are abundant among the polyacetylenes found in *P. ginseng* (Kim *et al*., 2022). Diacetyl alcohol, triacetyl alcohol, acetic acid, and linolenic acid are the polyacetylenes found in ginseng (Xu *et al*., 2011). These compounds have been isolated from fresh ginseng roots and

exhibit various biological activities, including cytotoxic effects on cancer cells which may contribute to therapeutic benefits such as promoting hair growth and potentially treating hair growth disorders like alopecia (Suzuki *et al*., 2017).

Table 2. Saponin and non-saponin components existing in Ginseng (Hyun *et al*., 2020)

Common components		
Saponin	Ginsenosides	Protopanaxadiol (27 types)
		Protopanaxatriol (14 types)
		Oleanane (2 types)
Non-saponin	Saccharides	Monosaccharide, disaccharide, trisaccharide, polysaccharides (including red ginseng polysaccharide, etc.), crude fiber, pectin
	Nitrogen-containing compounds	Protein, peptide, amino acid, nucleic acids, alkaloid
	Fat-soluble components	Lipid, fatty acid, polyacetylenes, phenolic compounds, essential oils, phytosterols, organic acid, terpenoid
	Vitamin	Water-soluble vitamins
	Ash	Minerals

2.3.5 MEDICINAL VALUE

The use of Chinese medicine has risen in popularity after the 2015 Nobel Prize was awarded for the discovery that artemisinin is an effective treatment for malaria (Tu *et al*., 2016). *ginseng* also known as Asian or Korean *ginseng*, is a medicinal herb traditionally used in Korea and China with a broad range of therapeutic properties largely attributed to its active compounds called ginsenoside. These compounds have been shown to exert antioxidation, anti-inflammatory, vasorelaxation, antiallergic, antidiabetic, and anticancer effects (Kim, 2018). *ginseng* and its derived natural products are amongst the most popular natural remedies and are used to treat various diseases and conditions such as diabetes, anti-oxidative, inflammation, cancers, fungal, bacterial, viral, stress and neurodegenerative diseases (Ahmad *et al*., 2023), as well as brain ischemia, hypertension, obesity, cardiovascular diseases and stroke, sarcopenia, muscle-wasting conditions, muscle aging, and cancer cachexia (Ahmad *et al*., 2023). Known side effects of *ginseng* include headaches, diarrhea, blood pressure changes, skin irritations, and vaginal bleeding (Ahmad *et al*., 2023). *ginseng* is known to produce numerous actions on the respiratory system, especially on asthma related with anti-allergic properties (Jung *et al*., 2013). Several studies have described the beneficial effects of *P. ginseng* on various psychiatric diseases such as depression and schizophrenia. It has been reported that *P. ginseng* has curative effects on depression by a plethora of studies (Park *et al*., 2018). Several studies have recently reported that *P. ginseng* has a wide range of actions in the central nervous system, with promising effects on Parkinson's disease. Van *et al.*

(2014) demonstrated neuroprotective effects of the *P. ginseng* extract. It significantly reduced dopaminergic cell loss, preventing the development of locomotor deficits in chronic Parkinson's disease model animals. Saponin and non-saponin compounds have been reported to show cytotoxic activities against various kinds of cancer cell lines in culture. Ginseng was found to have the ability to induce neoplastic cells into normal cells (Sang *et al*., 1983), aiding in the treatment of cancer.

Table 3: showing the different constituents of panax ginseng and their medicinal value (Jin *et al*., 2019).

Category	**Active ingredients**	**Effects**
Ginsenosides	Protopanaxadiols: Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2 Protopanaxatriols: Rg1, Rg2, Re, Rf, Rh1, Rh3 oleanolic acid: Ro	Anti-depression, anti-tumor, anti-ageing, anti-ischemic brain injury, immunomodulation, CNS regulation
Ginseng polysaccharides	Monosaccharide: glucose, galactose, arabinose, rhamnose, galacturonic acid, mannose e.t.c	Immunomodulation, anti-tumor, inhibition of liver injury, hypoglycemic activity

Category	Active ingredients	Effects
	Disaccharide: sucrose, maltose, locust, e.t.c. Polysaccharide: ginseng trisaccharide and ginseng tetrasaccharide	
Ginseng polypeptides	Oligopeptide I, II, II, IV	Hypolipidemic and hepatic glycogen-lowering
Volatile oils	Sesquiterpenes, β-elemene, panaxynol panaxydol, panaxytriol, ginsenyne, panasinsene, etc	Bacteriostasis and improvement of myocardial ischaemic injury
Polyacetylenes	Panaxydol, panaxytriol	Anti-tumour and anti-leukaemia
Organic acids and esters	Citric acid, isocitric acid, fumaric acid, linoleic acid, malic acid, succinic acid, tartaric acid, panax acid, triglyceride, palmitic acid, etc	Anti-oxidation
Others	Microelements, vitamins, alkaloids, lignins	Regulation of growth, metabolism and immune function

2.4 PHARMACOLOGICAL ACTIVITY/MECHANISM

Numerous studies have investigated the pharmacological activity/mechanisms of *ginseng*. Most of the studies have focused on the actions of ginsenosides, the major active component of *ginseng*. It is commonly believed that most pharmacological effects of *P. ginseng* are attributed to ginsenosides, including the stimulatory and inhibitory effects on the nervous system, antineoplastic effects, immunomodulatory effects, and nitric oxide release (Park *et al*., 2018). However, *ginseng* reportedly contains various potentially bioactive ingredients such as phytosterols, sesquiterpenes, flavonoids, polyacetylenes, alkaloids, and phenolic compounds in addition to ginsenosides (Kim, 2016), and these ingredients may also work together with ginsenosides to contribute to the various effects of *P. ginseng* (Kim, 2016).

Despite the previous efforts to understand the molecular mechanisms, it is still unclear how the combinations of multiple ingredients work together to produce clinical effects of *P. ginseng*. The conventional pharmacological approaches are unable to capture the systems-level mechanism of herbs. In a study by Park *et al* (2018), the systems-level mechanism of *P. ginseng* was reviewed by adopting network pharmacological analysis, providing new insights into the effects and mechanisms of *P. ginseng*. In the study, the systems-level mechanism of *P. ginseng* was reviewed by adopting novel analytical framework–network pharmacology. The targets of tnetwork were analyzed in terms of related biological process, pathways, and diseases. The majority of targets were found to be related with primary metabolic process, signal transduction, nitrogen compound metabolic process, blood circulation, immune system process, cell-cell signaling, biosynthetic process, and neurological system process, this showed the different mechanism by which panax ginseng works. In another study by Jin *et al*, (2019) on the mechanism of *panax ginseng* as an antidepressant, it was shown that through the metabolomics

in serum, urine and cerebral tissue of chronic unpredictable mild stress (CUMS) rats, ginsenosides were found to mitigate changes in amino acid neurotransmitters and monoamine neurotransmitters via modulation of neurotransmitter system. In another study, Liu *et al*, (2017) studied on the mechanism of *panax ginseng* on acetaminophen mediated live damage. In their study it was shown that Serum ALT and AST are the most sensitive biomarker enzymes used in the evaluation of acute liver injury. They observed significant increases in serum ALT and AST activities in APAP-induced mice compared with the normal group. The results in the present study showed that *panax ginseng* inhibited the serum levels via attenuation of oxidative stress and nitrative stress, suppression of inflammation and apoptosis.

In a nutshell the pharmacological activity/mechanism of panax ginseng can be summarized below.

2.4.1 Anti-apoptotic effects

Panax ginseng exerts its antiapoptotic effects by inhibiting key pro-death proteins and signaling pathways, enhancing cell survival signaling, and reducing oxidative and inflammatory damage, (Kim *et al*., 2013) making it a promising agent for protecting cells against various pathological injuries. It prevents apoptosis induced by stressors such as serum deprivation in neuronal cells (PC12 cells) through activation of protein kinase A (PKA) and atypical protein kinase C (PKCζ) pathways, which promote cell survival (Lin *et al.*, 2009).

2.4.2 Anti-oxidant effect

An antioxidant is a compound that reduces or scavenges reactive species or blocks the oxidation in cells (Bhatia and Tandon, 2005). It has been reported that P. ginseng and its derivatives are some of the antioxidant-rich sources involved in the regulation of many oxidative-stress-related pathways (He *et al*., 2022). *ginseng* and ginsenosides have antioxidant activity, inhibiting

oxidative stress, and their preventive effects are explained by their ability to scavenge ROS. Furthermore, the supplementation of P. ginseng increases antioxidant enzymes and reduces the generation of ROS and MDA in various tissues such as heart, lung, kidney, and liver in animal models (Morshed *et al*., 2023). According to growing researches, ginsenosides have a lot of potential to offer multiple strategies for treating different cancers (Morshed *et al.,* 2023).

2.4.3 Anti- inflammatory effect

Inflammation is part of the immunological response in the body to infection or injury, and it is associated with numerous human diseases and conditions (Ferrero *et al*., 2017). A dynamic balance between pro-inflammatory cytokines (TNF-α and IL-1β) and anti-inflammatory cytokines (IL-2, IL-4, and IL-10) modulates the status of inflammation, while an imbalance or overwhelming production of pro-inflammatory cytokines subsequently results in inflammation-related diseases such as diabetes, cancer, cardiovascular disease, and neurological diseases (Dong, 2020). The anti-inflammatory effects of ginseng extracts were proven with purified ginsenosides. The negative regulation of pro-inflammatory cytokine expressions (TNF-α, IL-1β, and IL-6) and enzyme expressions (iNOS and COX-2) was found as the anti-inflammatory mechanism of ginsenosides in M1-polarized macrophages and microglia (Kim *et al*., 2017).

2.5 TOXICOLOGICAL PROPERTIES OF *Panax ginseng*

Despite the worldwide use of traditional medicine, there have been concerns about the lack of safety information. An important role of safety is to identify the poison that induces the adverse effects involved in the interaction between toxicants and the cells (Bak *et al*., 2014). During the toxicity study of *ginseng* by Park *et al* (2022) *ginseng*-treated groups showed no noticeable abnormality in clinical signs, body weight gain, food and water consumption and urinalysis, hematological, serum biochemical and histopathological analyses did not find any BGE-related

toxicity (Park *et al* 2022). Known side effects of *ginseng* include headaches, diarrhea, blood pressure changes, skin irritations, and vaginal bleeding (Ahmad *et al*., 2023).500, 1,000, and 2,000 mg/kg/day for four weeks showed neither deaths nor clinical symptoms were observed (Park *et al*., 2013) However, the American Herbal Products Association, in their Botanical Safety Hand- book indicates that ginseng is a Class 2d herb (contraindicated for hypertension).

2.6 EFFECT OF *Panax ginseng* ON MALE FERTILITY ACTIVITY

Male infertility is a growing global concern, with conditions like oligospermia (low sperm count) and teratospermia (abnormal sperm morphology) being significant contributors to impaired fertility. These conditions often result in reduced reproductive potential, making it difficult for couples to conceive naturally. While various medical and lifestyle interventions exist to manage male infertility, there is increasing interest in alternative therapies, such as the use of herbal supplements. One such supplement that has been traditionally utilized for improving sexual and reproductive health is *Panax ginseng* (Lokesh *et al*., 2024). The active compounds in Ginseng, mainly ginsenosides, have been linked to improved sperm motility, morphology, and overall count, which could be particularly beneficial for individuals suffering from teratospermia and oligospermia (Lokesh *et al*., 2024). In terms of *ginseng's* effects on semen, numerous studies have shown that it improved semen quality in animal models. In rats, various ginsenosides have shown enhancing effects on sperm count and motility after treatment. Saponins from the cultured root of *P. ginseng* showed effects on spermatogenesis in male rats (Yun *et al*., 2018). *ginseng* is thought to exert its effects on male fertiliy through several mechanisms, including its powerful antioxidant properties, regulation of hormonal balance, and its ability to improve circulation, all of which may influence sperm health and function (Lokesh *et al*., 2024).

2.6.1 Hormone regulation

Ginseng has been shown to influence various hormones involved in spermatogenesis, including testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). *Ginseng* has been found to increase the secretion of testosterone by stimulating the hypothalamic-pituitary-gonadal (HPG) axis. This increase in testosterone can potentially help improve sperm count in men with oligospermia. Additionally, *ginseng* has been shown to influence the secretion of other reproductive hormones such as LH and FSH, further supporting its potential role in improving fertility (Lokesh *et al*., 2024).

2.6.2 Improvement of blood circulation

ginseng's ability to enhance blood circulation is another key factor that may contribute to its positive effects on sperm quality. Good blood flow to the testes is essential for the proper delivery of oxygen and nutrients, which support spermatogenesis. Ginseng's vasodilatory effect, which helps in the dilation of blood vessels and enhances blood flow, may improve oxygenation and nutrient supply to the testes, thus promoting better sperm production. This effect can be particularly beneficial for men suffering from poor testicular blood flow, which is often seen in oligospermia (Lokesh *et al*., 2024).

2.6.3 Promotion of spermatogenesis

ginseng's ginsenosides can enhance the proliferation of germ cells in the testes, thus promoting the process of spermatogenesis. By improving the function of Sertoli cells, which provide structural and nutritional support to developing sperm cells, *ginseng* may improve sperm count, motility, and morphology. This could be particularly beneficial for men with oligospermia, where sperm production is insufficient (Lokesh *et al*., 2024).

2.8 HEAVY METALS

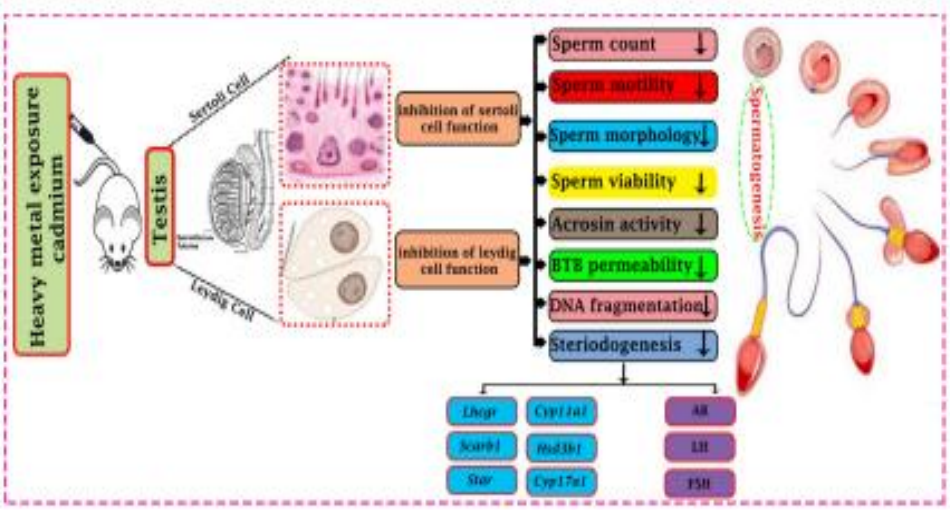
Heavy metals are Heterogenous group of elements varied in their functions and chemical properties. Heavy metals mainly belong to the transition element in the Periodic Table. They are either essential (Mo, Mn Cu, Ni, Fe, Zn) or non-essential metals (Cd, Ni, As, Hg, Pb). Essential metals maintain metabolism of human body like Cu is essential for hemoglobin formation, carbohydrate metabolism but if these are present in excess, it causes cellular damage. Deficiencies of essential heavy metals affect human health and agricultural productivities. Non-essential metals show toxic effect even at low concentrations (Kiran *et al*., 2022). They neither metabolized in other intermediate compounds nor break down in the environment. Due to the industrial, domestic, agricultural, medicinal, technological application or events like volcanic explosions and weathering of rocks, heavy metals release in the ecosystem. Heavy metals in ecosystem form a contamination chain in a cyclic manner-industry, atmosphere, soil, water, food and human (Kiran *et al*., 2022).

2.9 Cadmium exposure

Cadmium (Cd), first discovered as an impurity of zinc carbonate, is a group IIB element and is characterized as a heavy metal (Zhang and Reynolds 2019). Cd has the characteristics of strong accumulation, which can cause varying degrees of damage to the liver, kidney, and reproductive system, especially the male reproductive system (Cao *et al*., 2017; Gu *et al*., 2020; Niknafs *et al*., 2015). A majority of Cd is used for the production of batteries (83%), while the rest is used for alloys, coatings, plating, and a stabilizer for plastics (IARC, 2012) (Zhang and Reynolds 2019). Due to its wide use in commercial products and anthropogenic activities, the levels of Cd have increased within the environment, workplace, and food supply; therefore, the need for understanding exposure routes and toxicity is of great importance (Jarup and Akesson, 2009;

Mason, 2013). Besides occupational exposure to Cd, the general population can be exposed environmentally. Environmental Cd contamination can be the result of anthropogenic activities or natural changes, In nature, Cd compounds can exist in several phases and can be found in air, water and sediment (Zhang and Reynolds 2019). The continuous accumulation of Cd in the human body may account for multiple defects in male reproductive function. A previous study demonstrated that low doses of Cd (1–2 mg/kg/day) can cause histological damage to the testis without histological effects on other organs (Aktoz *et al*., 2010). In adult mice, 2 mg/kg/day Cd was injected intraperitoneally for 2 weeks, and the serum testosterone level was significantly lower than that of the control group (Minutoli *et al*., 2015). In vitro experiments also showed that Cd significantly increased the apoptosis of spermatogenic cells and decreasedthe number of spermatogenic cells (Angenard *et al*., 2010). Cd exposure damages testicular tissues and reduces testosterone levels, leading to male reproductive toxicity.

Figure 2.4 .Effect of cadmium toxicity on the functional activity of a male reproductive organ (Ali *et al*., 2022)



2.9.3 Histopathologic effect of cadmium toxicity

Approximately 15% of couples experience infertility which affects their life. Almost 50% of these cases are attributed to male factors, such as impaired spermatogenesis (Shojaeepour *et al*., 2021). In recent years, the study of Cd on male reproductive toxicity has gradually increased showing immerse implication on histopathologic features (Hu *et al*., 2015; Khanna *et al*., 2016; Mitra *et al*., 2016). In a study by Refaiy and Eissa, (2013) using an animal model to see the histopathologic effects of cadmium toxicity, the rats treated with Cd showed many histopathological changes; odema, degeneration of spermatogenic cells, pyknotic nuclei, and dilatation and congestion of blood vessels. In addition, Cd induced a pronounced alteration of the spermatogenic process with dramatical reductions of spermatozoa produced in the lumen of the seminiferous tubules sections. In another study by Shojaeepour *et al,* (2021), Cd-induced various damages in the testes with a variety of microscopic patterns, including sloughing and disorganization, incomplete spermatocytic arrest, spermatogenic hypoplasia, regional or incomplete fibrosis, tubular hyalinization, mixed atrophy, in addition to necrosis and calcification. These studies reveals the histopathologic effects of cadmium.

2.9.4 Cadmium induced testicular toxicity and its effect on spermatogenesis

Mammalian testis contains two compartments, the seminiferous epithelium (SCs bind together to support spermatogenesis), and the interstitial compartment, in which Leydig cells (LCs) secrete androgen and peptide hormone such as insulin-like 3 (INSL3) to regulate the development of the male reproductive tract, the descent of testis and the spermatogenesis (Zhu *et al*., 2020).

SCs play a critical role in the assembly of the testis cords during the fetal and neonatal periods. When the SCs in the testis of newborn mice are eliminated, the tubule structure is lost, and subsequent development of adult Leydig cells (ALCs) in the adult testis is severely blocked. In adult testes, SCs are essential for maintaining spermatogenesis, and elimination of the SCs in adult testes can lead to loss of germ cells (Rebourcet *et al*., 2014). Besides, in the fetus, SCs secrete anti-Müllerian hormone (AMH), which causes the regression of Müllerian duct. In the fetal life of rodents and humans, the number of SCs increases exponentially and then slows down after birth and reaches adult levels in early puberty (Zhu *et al*., 2020).

Cd affects SC development during fetal and neonatal periods. A single intraperitoneal injection (ip.) of low doses of Cd to rats on GD12 down-regulates the expression of SC genes (*Dhh* and *Fshr*), although this does not affect its number (Li *et al*., 2018). Exposure to Cd (1–2 mg/kg, sc) in pregnant and lactating rats can cause vacuolation of SCs and loss of germ cells in adult seminiferous epithelium. Cd inhibits proliferation and induces apoptosis and DNA damage of immature SCs in the piglet testis (Zhang *et al*., 2018). Cd inhibits the interaction between neonatal SC and gonocyte via p38 MAPK signaling in the SC-gonocyte co-culture system *in vitro* (Yu *et a*l., 2008).

Adult SCs are the target of Cd. Exposure of rats to Cd of 1 mg/kg daily by gavage for 28 days can cause severe ultrastructure changes in adult SCs. Rats exposed to a single dose of Cd (3 μmol/kg) show vacuolation in the SC cytoplasm and irregular chromatin condensation in late spermatids. Exposure to Cd by inhalation for 28 days can cause severe mitochondrial changes in SCs of adult mice

2.9.5 Effect of Cd on Sperm Development

Cd affects sperm development. Rats exposed to a single dose of (0.67–1.1 mg/kg) of Cd for 7 days show disorganization of the seminiferous epithelium (Cupertino *et al*., 2017). After 28 days of oral administration of Cd (5 mg/kg) for 28 days, rat's sperm count, motility, and viability decline (Nna *et al*., 2017). When rats are exposed to Cd (0.2 mg/kg, sc) for 15 days, the seminiferous tubules of their testes are disarranged, the number of germ cells decreases (Jahan *et al*., 2014). Adult male rats have significantly damaged seminiferous tubules after 56 days of exposure to Cd (1.15 mg/kg, i.p) (Leite *et al*., 2013). Cd (3 mg/kg, sc, once a week) exposure to rats for 4 weeks also contract seminiferous tubules and deplete germ cells and increase multinucleated giant cells (Rajendar *et al*., 2012).

2.9.6 Effects of Cd on Mature Sperm Function

Cd affects mature sperm function. After *in vitro* treatment with human and mouse sperm, Cd remarkably reduces sperm motility and progressive motility (Zhao *et al*., 2017). Short-term treatment of Cd (30 min) will not influence sperm motility, but significantly reduces the *in vitro* fertilization rate to egg and delays early embryonic development in mice, suggesting that Cd works epigenetically (Zhao *et al*., 2017). Cd also lowers human sperm motility and forward motility (Zhao *et al*., 2017).

2.9.7 Effect of Cadmium on Leydig Cells

The integrity of Leydig cells is important for spermatogenesis. Leydig cells are responsible for the secretion and production of androgen hormones. However, cadmium decreases the circulating testosterone, causes disorganization of mitochondria of Leydig cells, cell viability, increased lipid peroxidation, DNA damage, and damage to the testicular blood vessels. Laskey *et al* (2014). observed that decreased testosterone levels appeared before morphological changes in the testis after cadmium injection. In male fetuses and their testicular progeny, androgens facilitate the development of the internal and external genitalia; androgens promote the development of the vas deferens, seminal vesicles, and Wolff's duct in male mammals.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY LOCATION AND DURATION

This study was conducted at the Department of Medical Laboratory Science, (Histopathology Unit Laboratory), School of Basic Medical Sciences, College of Medical Sciences, University of Benin and the animal housing facility of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. The facility provided a controlled environment necessary for the experimental study, including standard housing conditions for laboratory animals and complied with institutional ethical standards and national regulations for the care and use of laboratory animals The study was carried for a total time frame of five weeks, with two weeks of acclimatization and 3 weeks of administration.

3.2 REAGENTS, CHEMICALS AND LABORATORY MATERIALS

Hematoxylin dye, eosin dye, 1% acid-alcohol, xylene, l0% Bouin's fluid, normal saline, alcohol, paraffin wax, 10% neutral buffered formalin, cadmium chloride, dissecting material, hot plate, microtome knives, digital weighing balance, cover slips, froosted end glass slide, pyrex glass wares, forceps, binocular microscope, cutup board, universal container, tissue cassette, rotary microtome, embedding machine, automatic tissue processor, floatation bath.

3.3 COLLECTION OF *ginseng*

ginseng tablet with NAFDAC Reg. No: A7-101718 was purchased from Hallmark supermarket at sapele road, Benin city, Edo state. Nigeria. It contained 90 tablets with each weighing 500mg, the tablets were crushed/grounded into powder and dissolved in distilled water and properly mixed.

3.4 PREPARATION OF THE HEAVY METAL

Cadmium chloride with batch no. L231151707 was purchased from LOBA CHEMIE PVT. LTD. 1mg of the cadmium chloride was weighed using a weighing balance, and was diluted using 10ml of distilled water.

3.5 ANIMAL CARE/ HOUSING

Twenty male Wistar albino rats weighing between 140g-200g was procured from the animal housing facility of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City. Animals were housed in plastic cages with wire gauge with a tripod that separates the animal from its feaces to prevent contamination and left to acclimatize for 14 days under standard conditions of temperature, and relative humidity 26ºc ± 2ºc and 50% respectively,

12:12hr natural light/dark cycle. The environment was kept clean, aerated and maintained regularly by the animal housekeeper and animals were handled according to international guidelines for handling experimental animals as reported by the Institute for Laboratory Animal Research (NRC, 1996). All animals were fed with standard pellets (standard feed Nigeria Plc.) and water ad libitum. Animals were frequently checked for signs of distress, weight loss, lack of appetite and given vetinary care.

3.6 ETHICS

Ethical approval was obtained from the Research Review Committee of the Ministry of Agriculture and Food Security, Edo state and an official approval number (MAFSAEC: 025-11/12/0049) was provided. All procedures involving animals conformed strictly to the guidelines for the care and use of laboratory animals and experimental rats were handled according to international guidelines for handling experimental animals as reported by the Institute for Laboratory Animal Research (NRC, 1996).

3.7 METHODOLOGY

3.7.1 EXPERIMENTAL DESIGN

The experimental design involved the use of animal model. Male wistar albino rat was used, the sum total of rat used was 20 rats, they were divided into 4 groups, each comprising of five rats.

Group I (n = 5 rats)

This group served as control. Rats were fed pelleted vita finisher feed and water for 3 weeks (21 consecutive days).

Group II (n =5rats)

This group served as negative control. Rats were administered Cadmium chloride 1mg/kg body weight intraperitoneally in addition to vita feed and water ad libitum for three weeks.

Group III (n = 5rats)

Rats were administered Cadmium chloride 1mg/kg intraperitoneally for 30mins, 200mg per kg body weight of *Panax ginseng* was administered. All animals were given the prescribed dosage orally using a syringe and gauge measuring 5 ml for three weeks.

Group IV (n = 5rats)

Rats were administered Cadmium chloride 1mg/kg for 30mins, 400mg per body weight per day of *Panax ginseng* was administered. All animals were given the prescribed dosage orally using a syringe and gauge measuring 5 ml for three weeks**.**

After receiving treatment for a total of three weeks, the animals were euthanized by cervical dislocation. Experimental rats testicular tissues were harvested, cleaned in normal saline, and then fixed in 10% Bouin fluid for histological examination.

DOSAGE CALCULATIONS

For cadmium

Using 1mg/kg body weight; Each rat was weighed

1mg = 1kg

1mg =1000g

Xmg = weight of rat in g

Xmg = 1mg x weight of rat (g)

1000g

Using 1g dissolved in 100ml

If 1000mg is dissolved in 100ml, Xmg will contain how many ml?

Therefore;

Xml = Xmg x 100ml

1000mg

The weight of each Rat was checked on a weekly basis to determine the new dosage for each Rat.

For *ginseng*

Using 200mg/kg body weight: each rat was weighed

200mg/kg= 1kg

200mg/kg = 1000g

Xmg = weight of rat in g

Xmg= 200mg weight of rat

1000g

Using 1000mg dissolved in 10ml

If 1000mg is dissolved in 10ml, Xmg will contain how many ml?

Therefore;

$$Xml = \frac{Xmg \times 10ml}{1000mg}$$

Note: The same calculation was done for group D (400mg/kg).

3.7.2. SEMINAL FLUID ANALYSIS

The sperm analysis involved sperm count, sperm motility, and sperm morphology and abnormal cells. Instruments like scissors, forceps, microscope, cover slips, slides, normal saline were used. The epididymis was surgically removed by anesthetizing the rat, shaving the abdominal area, and cleaning it with 70% ethanol. The semen was extracted from the epididymis avoiding damage to cells, a drop of normal saline was added and gently mixed, a drop of the mixture was placed on a greeze free glass slide, covered with a coverslip, and viewed under the microscope. Sperm abnormalities were identified by combining one drop of diluted semen with an Eosin stain, estimating the percentage of abnormal and normal sperms, and then dilution with formalin and sodium bicarbonate solution to estimate the concentration of epididymal sperm, followed by sperm counting with a Neuberger hemocytometer

3.7.3 SACRIFICING OF ANIMALS

At the end of the 3 weeks, animals were sacrificed 24 hours after last day of administration. Animals from each group were weighed and humanely euthanized using cervical dislocation, rats were anesthetized with kitamine. The testis was excised from experimental groups including the control with a sterile surgical blade, organ weight of each testis was taken and testis were fixed in 10% Boiuns fluid. Animal carcass were disposed by burial following guidelines for disposal of laboratory animals.

3.7.4 HISTOLOGICAL PROCEDURES

Grossing and processing

The harvested testicular tissues (testis) were examined macroscopically for ulceration and inflammation, and then grossed into slabs of 5mm thickness, immersed in 10% neutral buffered formalin for at least 24 hours at room temperature to achieve thorough penetration and preserve tissue architecture, preventing autolysis and minimizing artifact. Tissue was there after processed using an automatic tissue processor, the processing involved

- Dehydration: This was done by sequential immersion in graded alcohols (70%, 90%, and 95% ethanol), graded alcohol was done to prevent tissue distortation from direct impact of concentrated alcohol on tissue, this was followed by three rounds of absolute alcohol, each lasting 2 hours and using a volume 50–100 times the tissue volume.
- Clearing: The samples were cleared in two changes of xylene for 90 minutes each, removing residual alcohol and preparing the tissue for paraffin infiltration.
- Infiltration/Impregnation: Paraffin wax impregnation was performed at wax melting temperature, using tissue-to-wax ratios of 1:25–30, with two changes of molten wax,

each lasting 2 hours, to ensure complete infiltration. After processing, tissues were embedded in molten paraffin wax. The embedded tissue were trimmed and sectioned at 3–5 μm thickness using a rotary microtome.

Staining

Tissue sections were taken to water by dewaxing/deparaffinizing in two changes of xylene, each for 2 minutes, to remove paraffin wax. This was followed by rehydration of tissue through a series of descending concentration of alcohol, starting with absolute alcohol, 90% alcohol and finally 70% alcohol one minute each, and finally tissue was rinsed in water. After rehydration, the sections were stained with hematoxylin for 10 minutes to highlight nuclear structures. Excess stain was removed by brief rinsing in distilled water for 30 seconds, followed by differentiation in 1% acid alcohol for 15 seconds to enhance contrast. The slides were then rinsed/blued thoroughly in distilled water for 5 minutes to stop the differentiation process. Subsequently, the tissues were counterstained with 1% eosin for 5 minutes to visualize cytoplasmic and extracellular components and thereafter rinsed in running tap water for 30 seconds, it was then dehydrated through ascending grades of alcohol 70%, 90%, and 100% for 1 minute each. Dehydrated slides were cleared in two changes of xylene for 2 minutes each to remove alcohol and increase visibility. Finally, the sections were mounted using DPX mounting medium and examined microscopically under an objective lens to assess histological features (Archibong *et al.*, 2021).

3.7.5 MICROSCOPY AND PHOTOMICROGRAPHY

At X40 and X100 magnifications, sections were examined using an Olympus binocular microscope with an integrated illumination system. After that, the sections were

photomicrographed under a microscope with an Olympus trinocular microscope linked to a digital camera.

3.7.6 STATISTICAL ANALYSIS

The mean and standard deviation were used to express all weight results. Satistical analysis was assessed using one-way ANOVA followed by Tukey's HSD post hoc test.

CHAPTER FOUR

STATISTICAL ANALYSIS AND RESULT

Statistical analysis

The effects of cadmium exposure and *Panax ginseng* treatment on body weight, testicular weight, and semen quality parameters were evaluated statistically. Data were expressed as mean ± SEM, and group comparisons were assessed using one-way ANOVA followed by Tukey's HSD post hoc test. Statistical significance was considered at $p < 0.05$. The results are presented in Tables 4.1–4.4.

Table 4.1 Effect of *Panax ginseng* on Body Weight and Testicular Weights in Cadmium-Induced Testicular Toxicity in Male Wistar Albino Rats

Group	n	Initial weight (g)	Final weight (g)	Right testis weight (g)	Left testis weight (g)
Group A	4	166.75 ± 13.36^{a}	213.50 ± 16.04^{a}	1.33 ± 0.06^{a}	1.33 ± 0.06^{a}
Group B	2	171.50 ± 9.50^{a}	150.00 ± 16.00^{b}	0.55 ± 0.05^{bc}	0.55 ± 0.05^{bc}
Group C	3	161.33 ± 1.76^{a}	121.67 ± 14.45^{b}	0.45 ± 0.15^{b}	0.45 ± 0.15^{c}
Group D	2	172.00 ± 4.00^{a}	168.50 ± 23.50^{b}	0.70 ± 0.21^{c}	0.60 ± 0.25^{b}
P value		0.904	0.024*	0.007*	0.013*

Values are expressed as mean ± SEM. Means with different superscript letters (a, b, c) within the same column are significantly different at $p < 0.05$ according to Tukey's HSD test.

The initial body weights did not differ significantly among the groups ($p = 0.904$). Final body weight showed a significant reduction ($p = 0.024$). Group A was significantly higher than Groups B, C, and D, while no significant differences were observed among Groups B, C, and D.

Right and left testis weights also differed significantly ($p = 0.007$ and $p = 0.013$, respectively). Group A was significantly heavier than Groups B, C, and D. Group C was significantly different from Group D, while Group B did not differ significantly from either Group C or Group D.

Table 4.2. Effect of *Panax ginseng* on Sperm Count in Cadmium-Induced Testicular Toxicity in Male Wistar Albino Rats ($\times 10^{6}$ cells/mm^{3})

Group	Mean ± SEM	Min–Max	n	P value
Group A	783.5 ± 18.3[a]	729–805	4	< 0.001
Group B	306.0 ± 2.0[b]	304-308	2	
Group C	310.5 ± 3.5[b]	307–314	2	
Group D	422.5 ± 27.5[c]	495–550	2	

Values are expressed as mean ± SEM. Means with different superscript significantly different at $p < 0.05$ according to Tukey's HSD test.

Sperm count varied significantly ($p < 0.001$). Group A recorded the highest count, significantly greater than Groups B, C, and D. Groups B and C did not differ from each other, but both were significantly lower than D.

Table 4.3. Effect of *Panax ginseng* on Progressive Sperm Motility in Cadmium-Induced Testicular Toxicity in Male Wistar Albino Rats (%)

Group	Mean ± SEM	Min–Max	n	P value
Group A	75.8 ± 1.9[a]	71–80	4	0.00018
Group B	35.0 ± 5.0[b]	30–40	2	
Group C	35.0 ± 5.0[b]	30–40	2	
Group D	47.5 ± 2.5[c]	45–50	2	

Values are expressed as mean ± SEM. Means with different superscript letters (a, b, c) are significantly different at $p < 0.05$ according to Tukey's HSD test.

Progressive sperm motility showed significant differences among groups ($p = 0.00018$). Group A had the highest motility, significantly higher than Groups B, C, and D. Groups B and C were statistically similar but significantly lower than D. Group D showed intermediate motility, significantly lower than A but higher than B and C.

Table 4.4. Effect of *Panax ginseng* on Sperm Morphology in Cadmium-Induced Testicular Toxicity in Male Wistar Albino Rats

Parameter	Mean ± SEM	Min–Max	n
Normal Forms	86.9 ± 4.1	56–98	11
Abnormal forms	13.1 ± 4.1	2–44	11

Normal sperm forms constituted 86.9 ± 4.1%, while abnormal forms made up 13.1 ± 4.1%

MORTALITY RECORDED: A total of seven mortality was recorded across all groups

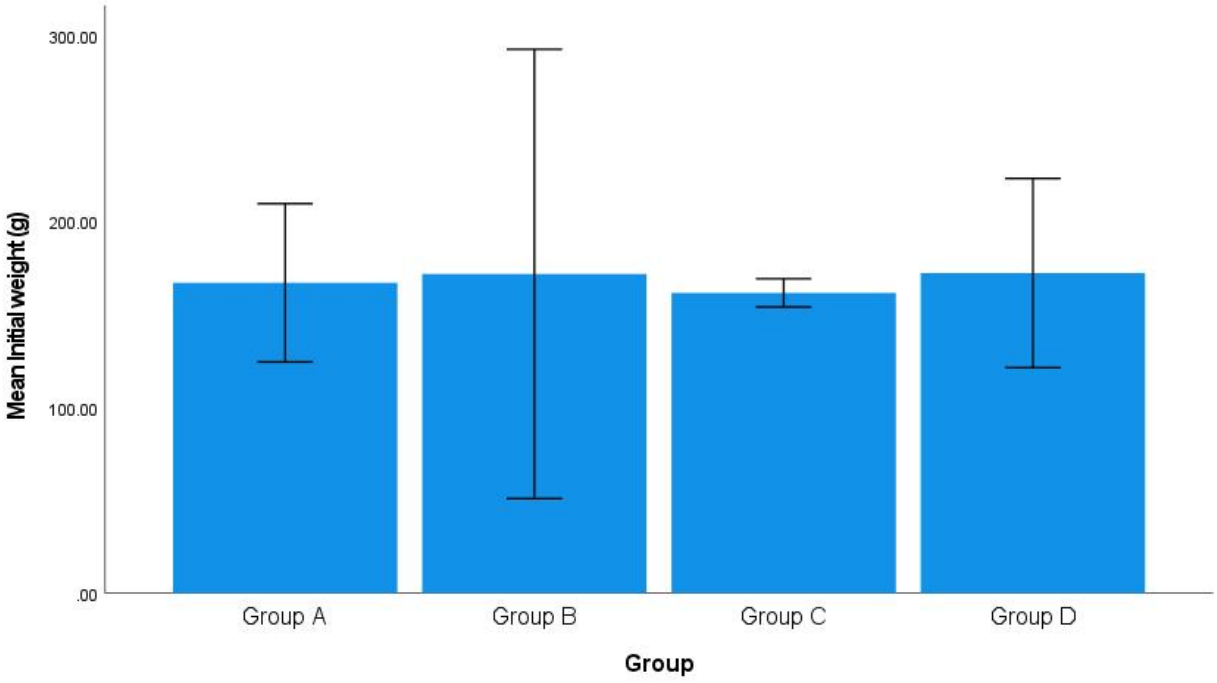


Figure 4.1 Mean Initial weight of Male Wistar Albino Rats

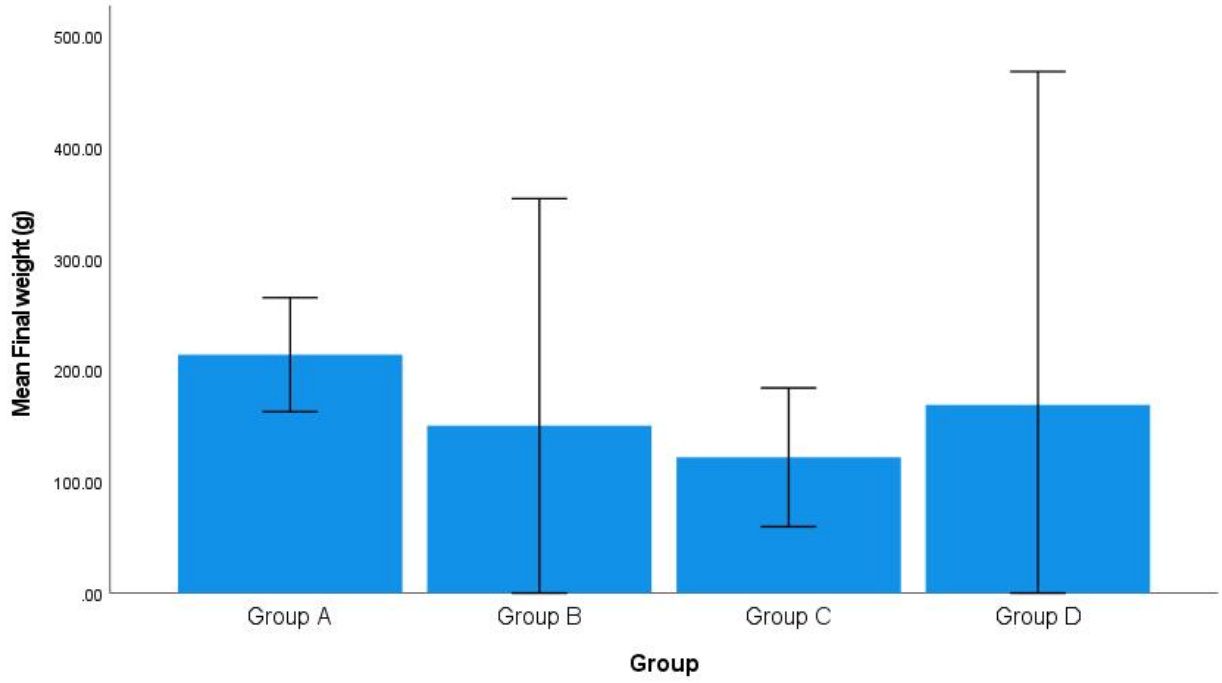


Figure 4.2 Mean final weight of Male Wistar Albino Rats exposed to Cadmium

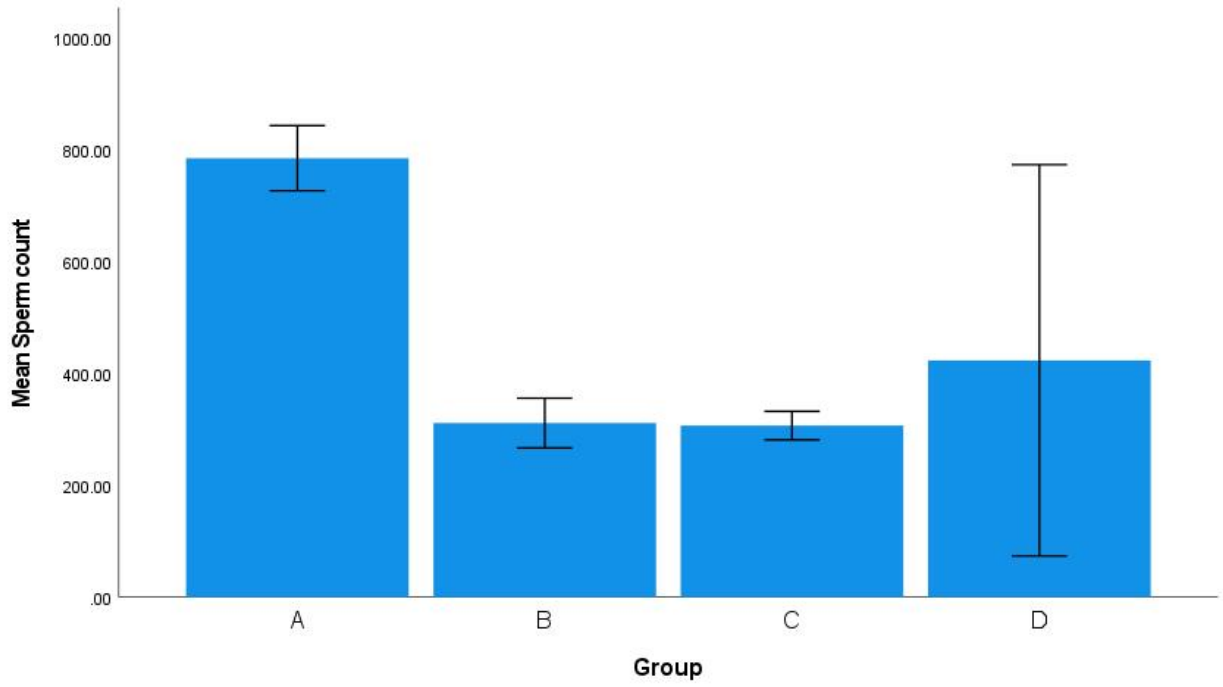


Figure 4.3 Mean sperm count of Wistar Albino Rats exposed to Cadmium

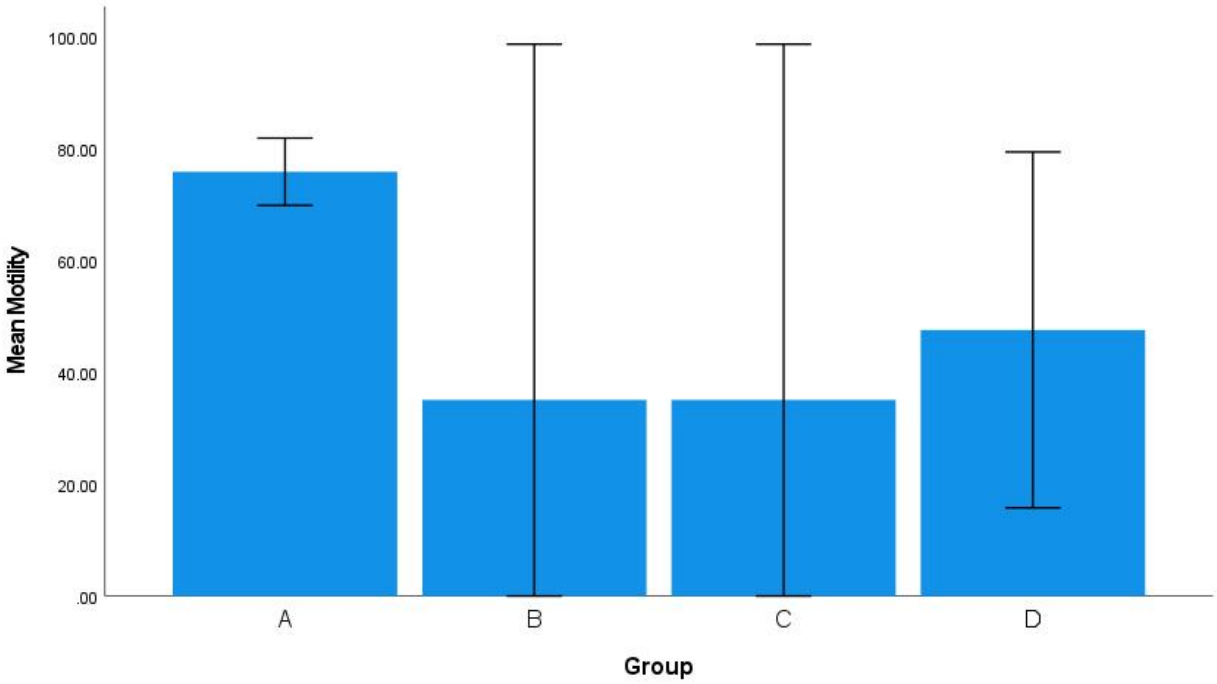

100.00
80.00
60.00
40.00
20.00
.00
Mean Motility
A
B
C
D
Group

Figure 4.4 Mean sperm motility rate of Wistar Albino Rats exposed to Cadmium

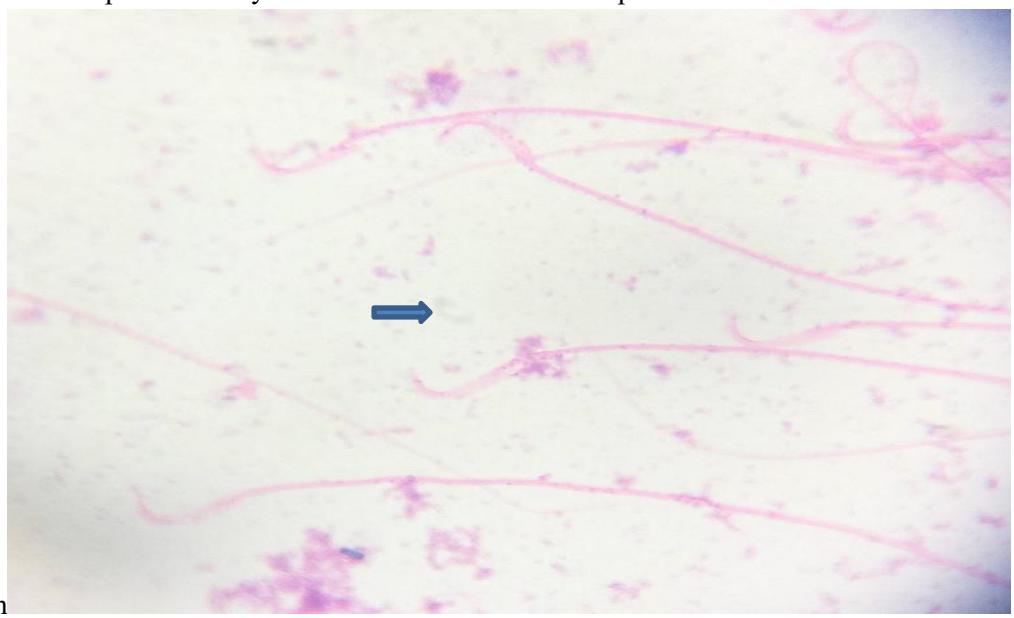


Plate 4.1: Photomicrograph of control group sperm cell with formed sperm cell showing head, neck and tail (thin arrow)

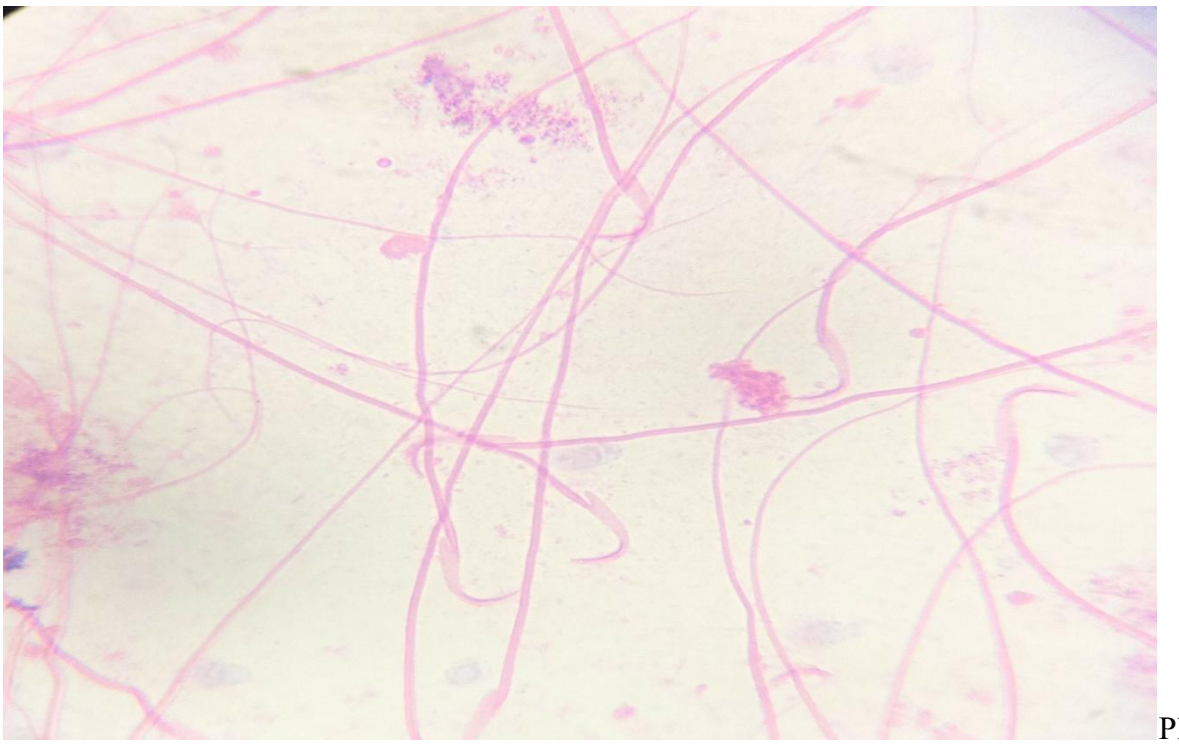


Plate 4.2: photomicrograph of group B showing sperm cell with formed sperm cell showing head, neck and tail (thin arrow) and a bent sperm cell (thick sperm cell)

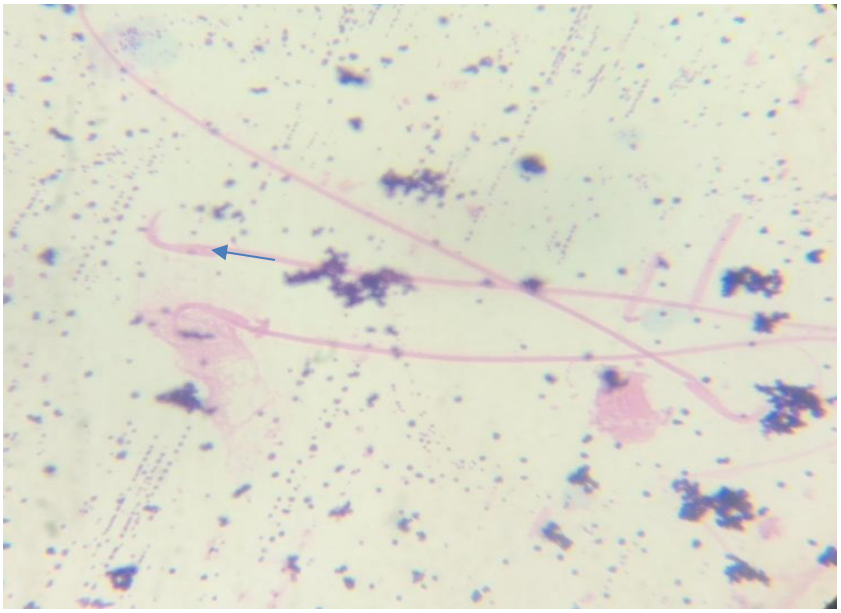


Plate 4.3: photomicrograph of group C showing sperm cell with formed with head, neck and tail (thin arrow)

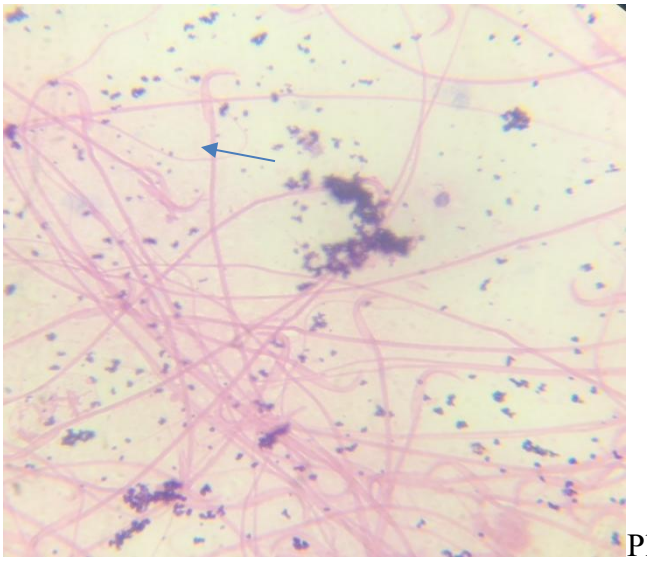


Plate 4.4: photomicrograph of group D showing sperm cell with formed sperm cell showing head, neck and tail (thin arrow)

4.2 Histopathological Changes

The histopathological alterations observed in the testes of male Wistar albino rats following cadmium exposure and subsequent treatment with *Panax ginseng* are presented in the photomicrographs below.

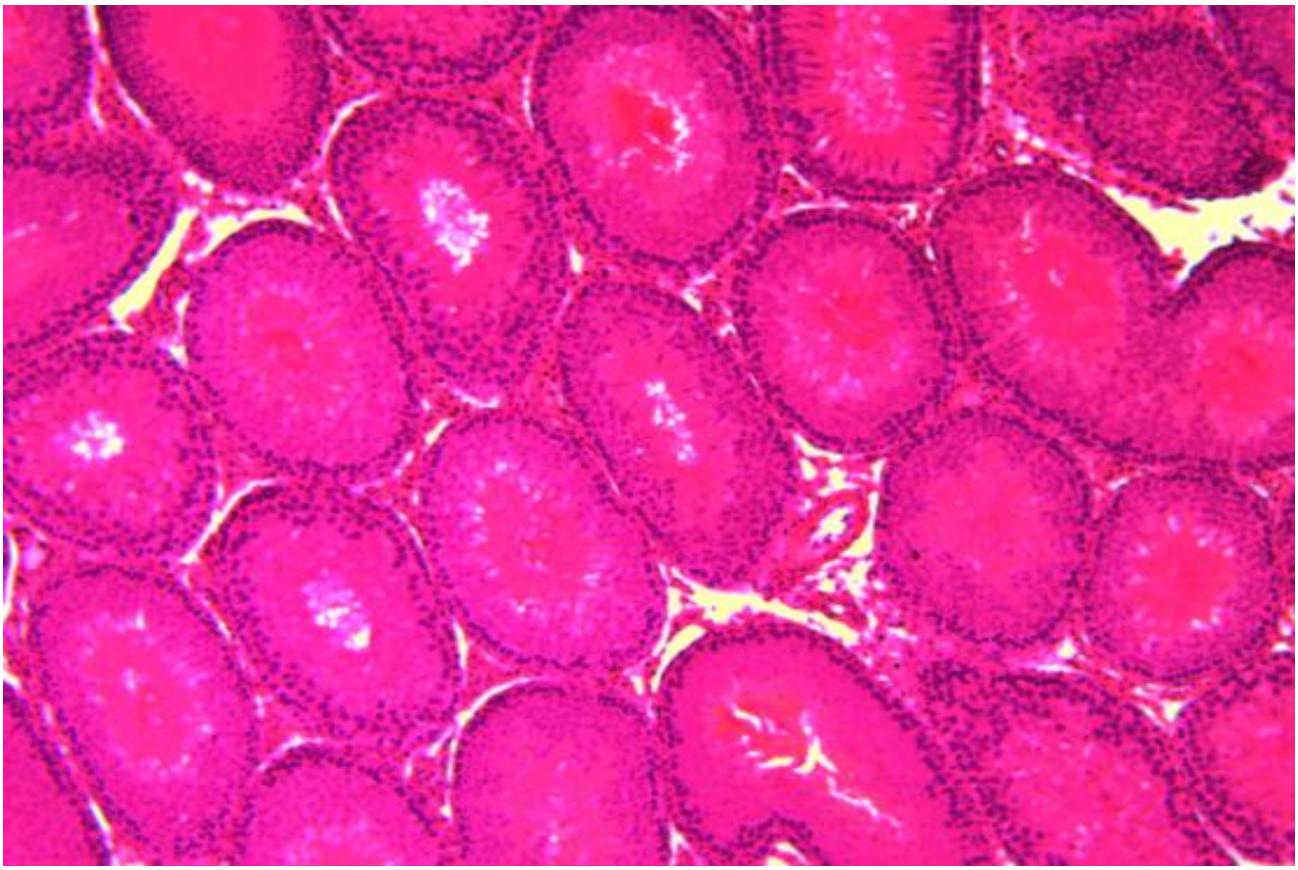
GROUP A (CONTROL): Sections of the testicular tissue from this group shown oval shape tubules, sperm cell at different maturation level and leydig cells, the testicular tissue were in keeping with healthy/normal testes. This is attributed to the fact that animal in this group were not exposed to toxicant or treatment but received only water and feed ad libitum.

GROUP B: Sections from this group showed shrunken seminiferous tubules with thickened basement membrane, no germ cells seen but only a mass of eosinophilic debris. This can be attributed to the exposure of animals to toxicant (cadmium chloride). Testicular tissue was in keeping with testicular atrophy.

GROUP C *ginseng* TREATED GROUP (200mg/kg/day): Sections from this group showed shrunken seminiferous tubules with thickened basement membrane, no germ cells seen but only a mass of eosinophilic debris, Leydig cell appeared normal. This can be attributed to the exposure of animals to toxicant (cadmium chloride). However, animals were treated with *ginseng*, this reveals treatment at this dosage was not effective enough. Testicular tissue were in keeping with testicular atrophy.

GROUP D *ginseng* TREATED GROUP (400mg/kg/day): Sections of the testicular tissue from this group shown oval shape tubules, sperm cell at different maturation level and leydig cells, the testicular tissue were in keeping with healthy/normal testes. This reveals that treatment at this dosage exerted protective effect against cadmium toxicity.

GROUP A X40 magnification



GROUP A X100 magnification

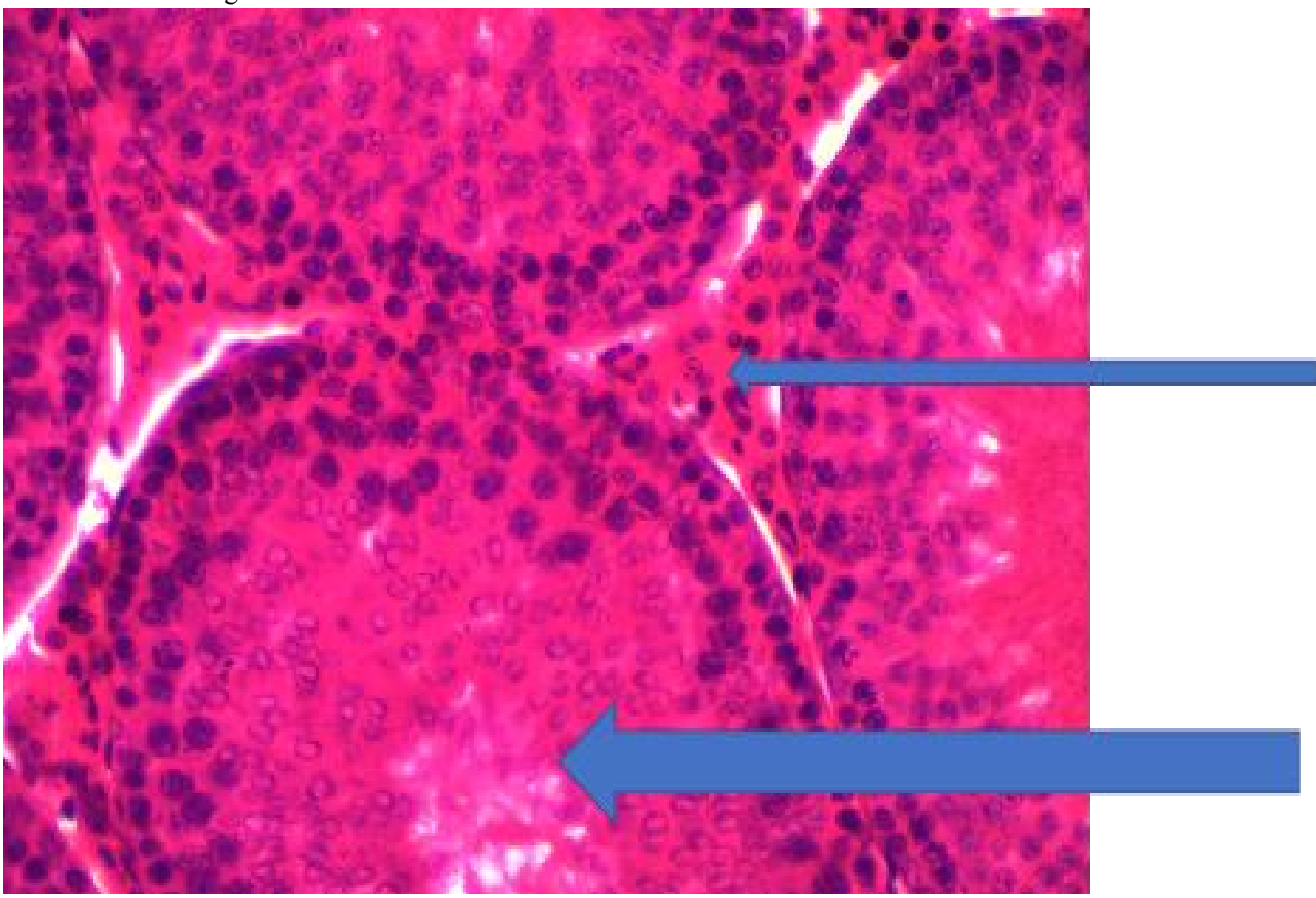
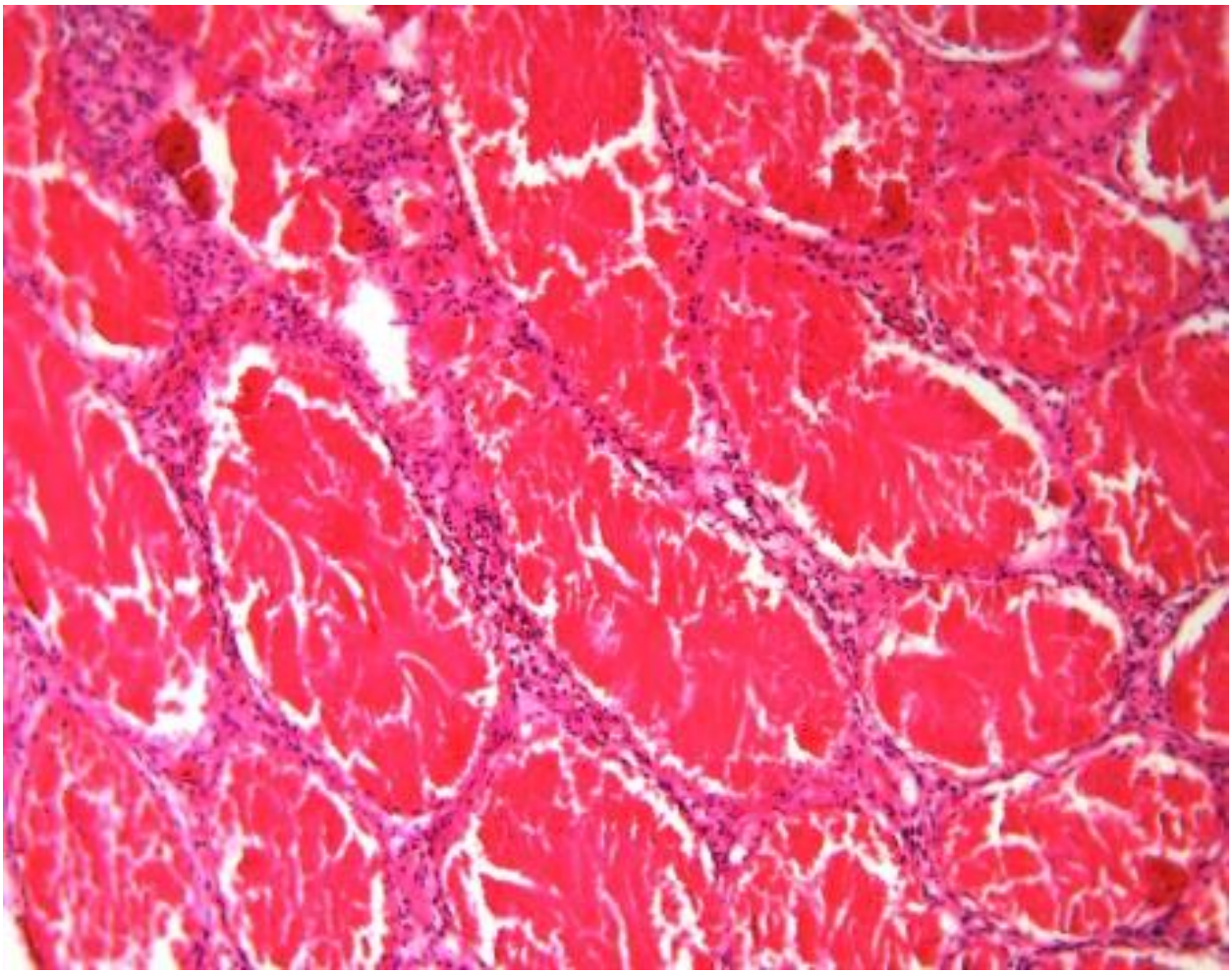


Plate 4.5. Photomicrograph of testis from control group showing oval seminiferous tubules (thick arrow) with Sertoli and germ cells at different maturation stages.

Section of the testis shows oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. FEATURES ARE IN KEEPING WITH NORMAL TESTIS

GROUP B X40 magnification



GROUP B X100 magnification

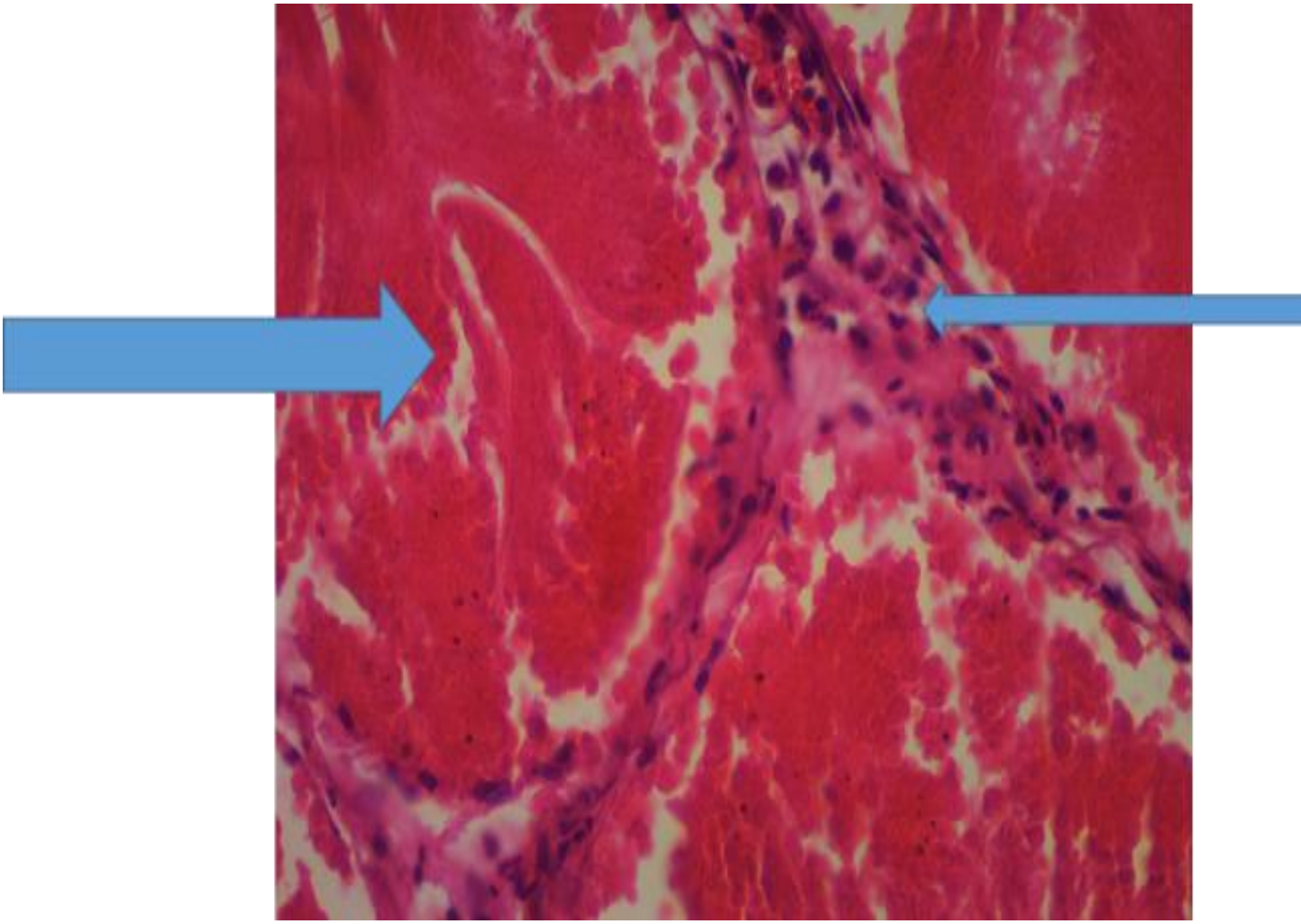
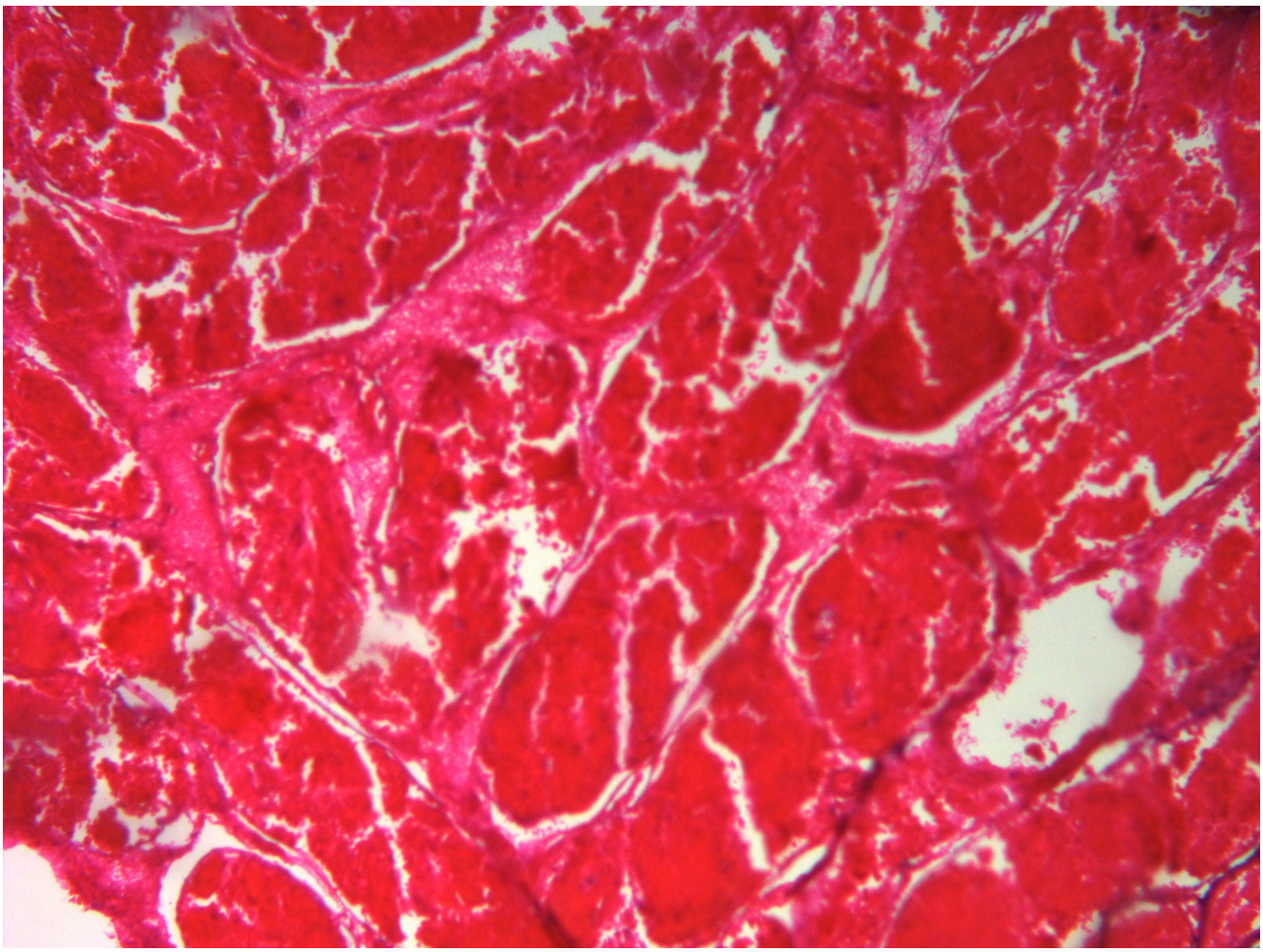


Plate 4.6. Photomicrograph of the Testis from Group B Showing Shrunken Seminiferous Tubules with Thickened Basement Membrane.

Section of the testis shows seminiferous tubules (thick arrow) that appears shrunken with thickened basement membrane. The tubules contain no germ cells but only a mass of eosinophilic debris. The Leydig cells (thin arrow) appears normal. FEATURES ARE IN KEEPING WITH TESTICULAR ATROPHY

GROUP C X40 magnification



GROUP C X100 magnification

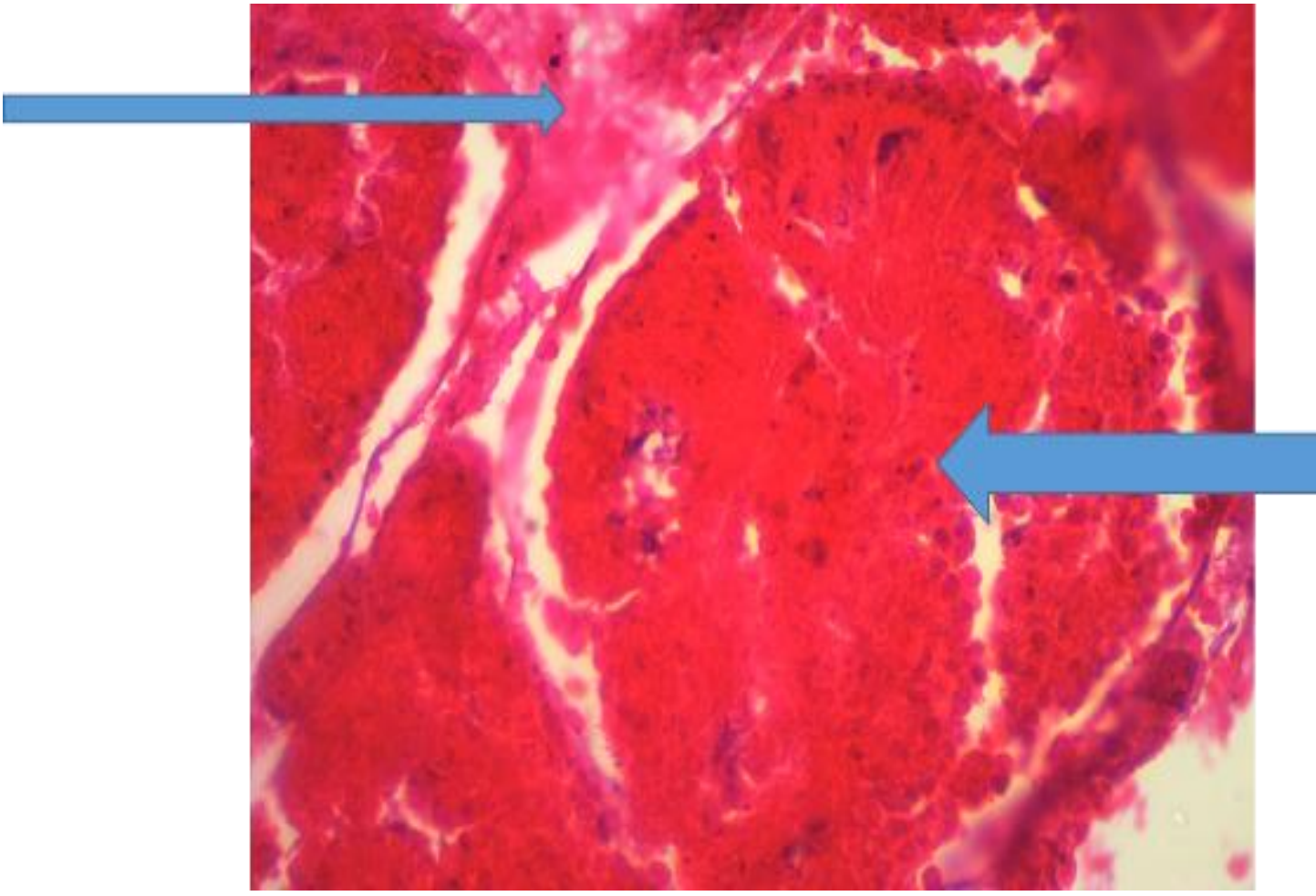
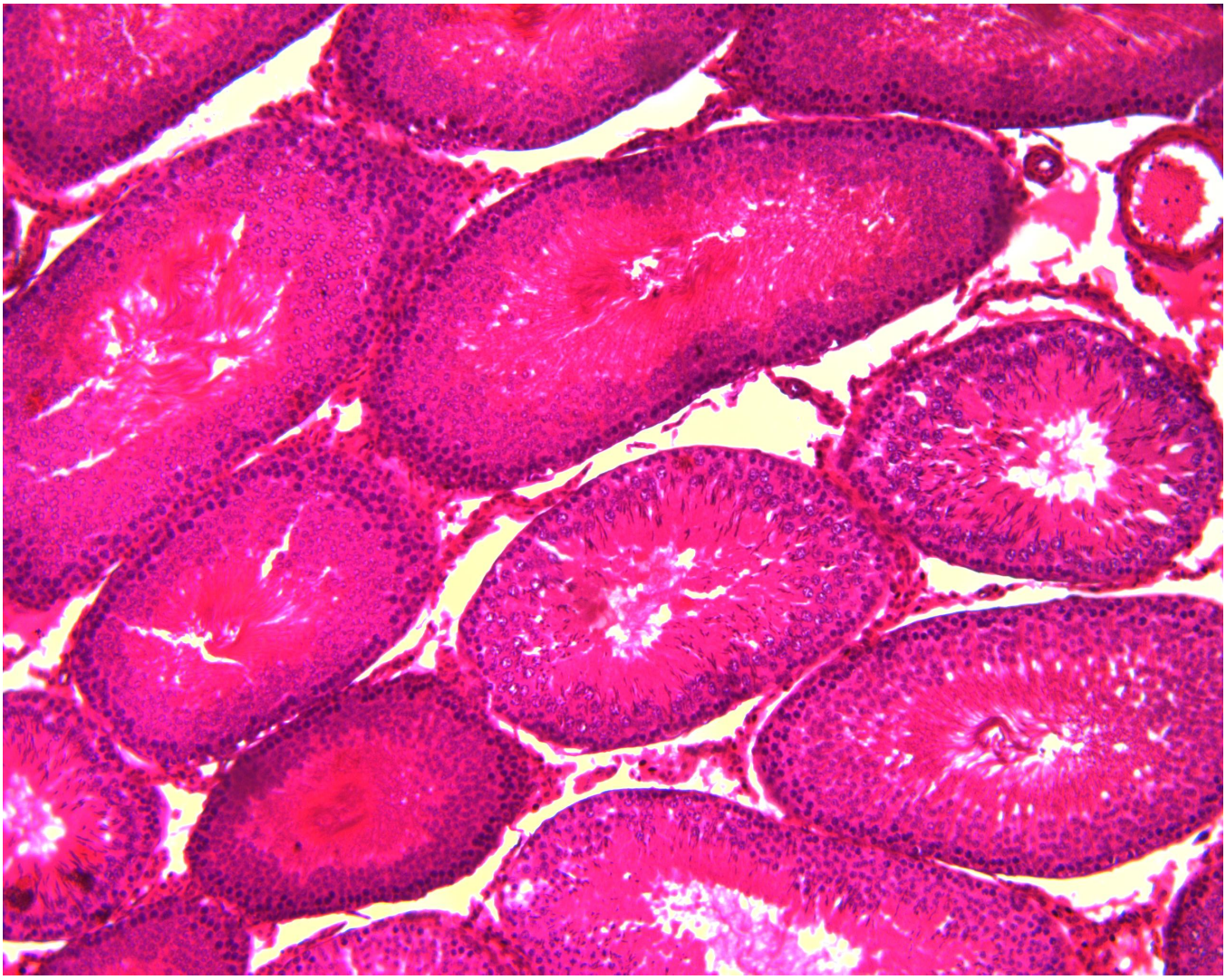


Plate 4.7. Photomicrograph of the Testis from Group B Showing Shrunken Seminiferous Tubules with Thickened Basement Membrane.

Section of the testis shows seminiferous tubules (thick arrow) that appears shrunken with thickened basement membrane. The tubules contain no germ cells but only a mass of eosinophilic debris. The Leydig cells (thin arrow) appears normal. FEATURES ARE IN KEEPING WITH TESTICULAR ATROPHY

GROUP D X 40 magnification



GROUP D X 100 magnification

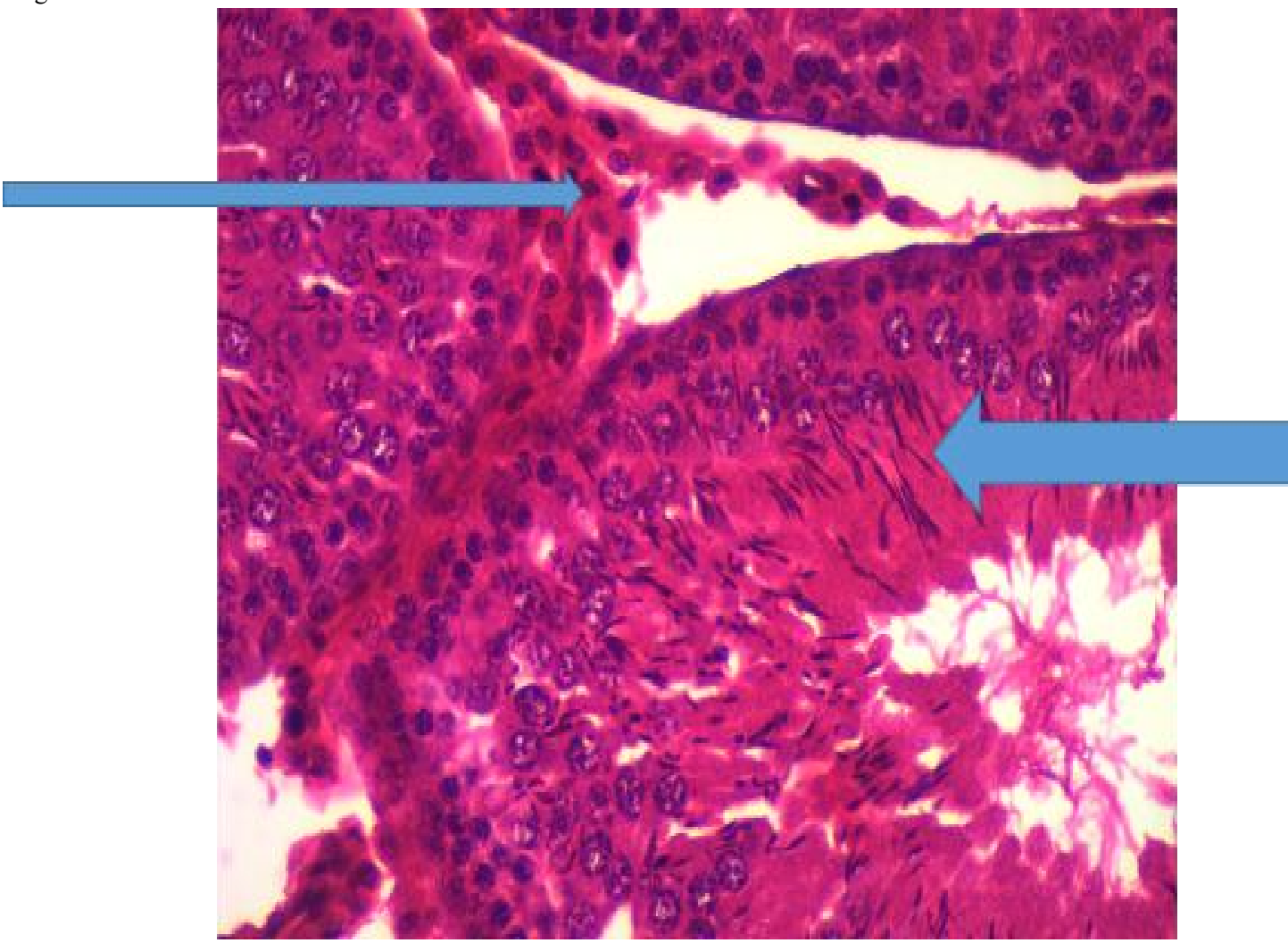


Plate 4.8 Photomicrograph of the Testis from Group D Showing Oval Seminiferous Tubules with Spermatogenic Cells and Normal Leydig Cells.

oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. **FEATURES ARE IN KEEPING WITH NORMAL TESTIS**

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

DISCUSSION

Infertility rate continues to trend higher in this century and about 15% of couples are infertile. Male causes of infertility account for 40–50% (Zhu *et al*., 2020) Cadmium negative impact on the male fertility has been documented by several studies like Khan *et al*. (2022). Because of this, the current study was conducted to examine the potential protective effect of *ginseng* against testicular toxicity caused by cadmium. The findings revealed that initial body weights did not differ among the groups ($p = 0.904$), but final body weight showed a significant reduction ($p = 0.024$). Group A (control) had higher weight compared to Groups B, C, and D which received cadmium, while no significant differences were observed among Groups B, C, and D. These findings contrast with the work of Smith *et al*. (2019), who found that while cadmium exposure affected certain physiological measures, it did not always result in significant changes in body weight. However, this study align with Jones and Lee. (2020), who reported that cadmium exposure in rats significantly reduced body weight due to toxicity. This discrepancy could be attributed to differences in experimental design, species, or duration of administration used in the studies.

The testicular weight in this study shows that group A had the highest value of left testis while group B had the lowest value, similarly, the right testis showed significant value ($P=0.007$). These findings may intardem with the findings of Brown *et al*. (2018) and Nguyen *et al*. (2021) which revealed that cadmium exposure leads to atrophy and reduced weight of the testes in rodents, likely due to its disruption of androgenic function and spermatogenesis. Reduction in

Testicular weight is a widely recognized and reliable biomarker for assessing reproductive toxicity, as it reflects both the structural and functional integrity of the testes. A reduction in testicular mass often signifies underlying histopathological damage, disruption of spermatogenesis, and hormonal imbalance (Liu *et al*., 2021). Since the testes are composed predominantly of germ cells and seminiferous tubules, any loss in their cellularity or impairment in spermatogenic activity can directly translate into decreased organ weight. The significant differences observed in this study add to the growing body of evidence that cadmium adversely affects testes weight, which could reflect impairment in reproductive health.

The significant variation in sperm count observed across the experimental groups ($p = 0.001$) highlight the profound impact of cadmium exposure on male reproductive health and the potential protective role of *Panax ginseng*. Group A (control) demonstrated the highest sperm count, which is expected in the absence of toxic exposure, reflecting normal spermatogenic activity. Interestingly, Group D, which received the higher dose of *ginseng* (400 mg/kg), exhibited sperm counts that was lower than the control but higher compared to the cadmium-only (group B) and group C(200mg/kg). This finding suggests that *Panax ginseng* has dose-dependent protective effects against cadmium-induced testicular damage, consistent with the report of Shojaeepour *et al*. (2021), who demonstrated that ginseng ameliorates cadmium-induced reproductive toxicity through its potent antioxidant and anti-apoptotic properties. Group B, which was exposed to cadmium without any *ginseng* treatment, exhibited the lowest sperm count. This aligns with the findings of Khan *et al.* (2022), who reported that cadmium exposure significantly reduces sperm count by inducing oxidative stress, disrupting the blood–testis barrier, and impairing the function of Sertoli and Leydig cells. Cadmium is known to increase the generation of reactive oxygen species (ROS), which exert reduced testicular antioxidant defenses,

leading to lipid peroxidation, DNA damage, and apoptosis of germ cells. These events culminate in reduced spermatogenesis and subsequent declines in sperm count.

Progressive sperm motility showed significant differences across groups ($p = 0.00018$). Group A had the highest motility (75.8 ± 1.9) significantly higher than Groups B, C, and D. Groups B and C recorded marked reduction in motility due to cadmium toxicity, Zhang *et al*. (2017) observed similar reductions in sperm motility following cadmium exposure, supporting the differences observed. Group D showed intermediate motility, significantly lower than A but noticeably higher than B and C. This is quite important as *panax ginseng* shows a level of protection in group D(400mg/kg) compared to group B and C. Since motility of sperm plays a crucial role in male fertility, impaired motility from cadmium is detrimental. The decline have been attributed to oxidative stress, mitochondria dysfunction, ROS generation. Another observation was the alteration in Sperm morphology seen in cadmium exposed group, ginseng protective effect was revealed in treated group, helping in alleviating cadmiums toxic effect on sperm morphology via hormone regulation, influence on spermatogenesis. The morphology of sperm is of great importances in male reproductive health, abnormal sperm morphology may result from altered sperm formation from toxicants like cadmium. Kaur *et al.* (2020) reported similar findings, where cadmium exposure led to decreased normal sperm morphology in rats, highlighting the toxic impact of cadmium on spermatogenesis, sperm structure.

Further-more, supporting the sperm parameters were the histological observations, which showed cadmium effects on the rat testicular tissue. In line with previous research, rats exposed to a dose of (0.67–1.1 mg/kg) show damage and thickening of the seminiferous epithelium (Zhu *et al*., 2020), decrease in the number of germ cells, accumulation of degenerated cellular debris in seminiferous tubules with atrophy (Jahan *et al*., 2014). Recently, the usage of herbal

medicines to enhance male functioning has gained popularity due to their antioxidant properties and capacity to promote sexual hormones (Fahmy *et al*., 2025). Since ancient times, *ginseng*, one of the most well-known medicinal herbs in the world, has been utilized in traditional Chinese medicine (Fahmy *et al*., 2025). Based on the obtained results, the co-administration of *ginseng* to cadmium toxicity, enhanced the testicular function and improved the male rat's fertility by marked improvement of the testicular histopathological architecture as shown by the photomicrograph of group D revealing oval seminiferous tubules with spermatogenic cells and normal leydig cells. This is evidence of the anti-apoptotic and anti-inflammatory activities of *ginseng* through the effective alleviation of seminal vesicle and testicular histopathological alterations (Fahmy *et al*., 2025).

CONCLUSION

This study findings on sperm parameters and histological examination shows that *panax ginseng* exhibit moderate protective effect against cadmium toxicity on sperm count, morphology, motility, and histological damage, this is in keeping with existing literature on its effect in alleviating cadmium testicular toxicity. Cadmium's effect was significantly shown in group that received cadmium and low dosage of panax ginseng, revealing that cadmium exposure disrupts testicular integrity, potentially due to its oxidative stress-inducing properties, which can damage sperm DNA and the overall integrity of sperm cells. *ginseng* at dosage 400mg/kg improved testicular function to a considerable amount due to its antioxidant, anti- inflammatory and anti-apoptotic properties combination therapy.

RECOMMENDATIONS

Investigations should focus on optimizing the dosage and treatment duration of ginseng to maximize its therapeutic benefits. Determining the most effective concentration and frequency of administration will be critical for ensuring its efficacy and safety in practical applications.

Regulatory approvals and public awareness initiatives are essential to facilitate the integration of *ginseng* into mainstream therapeutic regimens. Approval from relevant agencies and targeted awareness campaigns could promote its use as a natural remedy for managing reproductive toxicity, highlighting its benefits and safety for wider acceptance

Expanding the scope of research should be considered. Clinical trials and comparative studies of the herbal and synthetic antioxidants should be conducted to validate the findings of this study and assess the potential for human application. These trials would help establish its place in integrative medicine and broaden its therapeutic applications.

REFRENCES

Ahmad, S. S., Ahmad, K., Hwang, Y. C., Lee, E. J., & Choi, I. (2023). Therapeutic Applications of Ginseng Natural Compounds for Health Management. *International Journal of Molecular Sciences*, *24*(24), 17290.

Alli, B. O. (2019). Cadmium: Health hazards and risks to workers. Journal of Occupational Health, 61(1), 3-11.

Ali Waseem, Ma Yonggang, Zhu Jiaqiao, Zou, Hui & Liu, Z. (2022). Mechanisms of cadmium-induced testicular injury: A risk to male fertility. *Cells*, 11(22), 3601.

Arunkumar, A., & Anand, R. (2016). Effect of Panax ginseng on cadmium-induced testicular toxicity in rats. *International Journal of Pharmacology and Clinical Sciences*, 5(1), 23–28.

Barakat, N., Alkhen, M. A., Khater, Y., & Khirallah, S. M. (2025). Effect of melatonin and ginseng on rat testis and sperm quality against cadmium toxicity via inhibiting oxidative

stress and autophagy pathways. *Journal of Trace Elements in Medicine and Biology, 88*, 127614.

Bak, M. J., Kim, K. B., Jun, M., & Jeong, W. S. (2014). Safety of red ginseng oil for single oral administration in Sprague-Dawley rats. *Journal of Ginseng Research, 38*(1), 78–81.

Cesare, M., & Rosaria, S. (2017). Panax ginseng and Panax quinquefolius: From pharmacology to toxicology. *Food and Chemical Toxicology, 107*(A), 362–372.

Cao, Z., Fang, Y. and Lu, Y. (2017). Melatonin alleviates cadmium-induced liver injury by inhibiting the TXNIPNLRP3 inflammasome. *Journal of Pineal Research*, 6(2):1-12

Choi, K. T. (2008). Botanical characteristics, pharmacological effects and medicinal components of Korean Panax ginseng C. A. Meyer. *Acta Pharmacologica Sinica, 29*(9), 1109–1118.

Cho, I. H. (2012). Effects of Panax ginseng in neurodegenerative diseases. *Journal of Ginseng Research, 36*(4), 342–353.

de Souza Predes, F., Diamante, M. A. S., & Dolder, H. (2010). Testis response to low doses of cadmium in Wistar rats. *International Journal of Experimental Pathology*, 91(2), 125–131.

Ding, M., Cheng, H., Li, X., Li, X., Zhang, M., Cui, D., Yang, Y., Tian, X., Wang, H., & Yang, W. (2024). Phytochemistry, quality control and biosynthesis in ginseng research from 2021 to 2023: A state-of-the-art review concerning advances and challenges. *Chinese Herbal Medicines, 16*(4), 505–520.

Dong-Soon, I. (2020). Pro-resolving effect of ginsenosides as an anti-inflammatory mechanism of Panax ginseng. *Biomolecules, 10*(3), 444.

El-Wakf, A. M., Hassan, H. A., Mahmoud, M. M., Habza, M. N., & Ali, F. A. (2012). Protective effect of Panax ginseng against cadmium-induced testicular toxicity in male rats. Experimental and Toxicologic Pathology, 64(5), 435–441

El-Refaiy, A. I., & Eissa, F. I. (2013). Histopathology and cytotoxicity as biomarkers in treated rats with cadmium and some therapeutic agents. *Saudi Journal of Biological Sciences, 20*(3), 265–280.

Giuseppe Genchi., Sinicropi Maria Stefania., Lauria, Graziantonio., Carocci, A., & Catalano, A. (2020). The effects of cadmium toxicity. *International Journal of Environmental Research and Public Health*, 17(11), 3782.

Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., & Groneberg, D. A. (2006). The toxicity of cadmium and resulting hazards for human health. *Journal of Occupational Medicine and Toxicology*, 1, 22.

Gu, J., Ren, Z. and Zhao, J. (2020). Calcimimetic compound NPS R-467 protects against chronic cadmium-induced mouse kidney injury by restoring autophagy process. *Ecotoxicology and Environmental Safety*, 18(9):1-12.

Hemalatha, D., & Hari, R. (2015). Protective effect of Panax ginseng extract against cadmium-induced oxidative stress in testes of albino rats. International Journal of Pharmacy and Pharmaceutical Sciences, 7(2), 352–356.

Hanbing, L., Xiaoyan, L., Yang, H., & Xiaohui, F. (2020). Chemical constituents of Panax ginseng and Panax notoginseng explain why they differ in therapeutic efficacy. *Pharmacological Research, 161*, 105263.

He, B., Chen, D., Zhang, X., Yang, R., Yang, Y., Chen, P., & Shen, Z. (2022). Oxidative stress and ginsenosides: An update on the molecular mechanisms. *Oxidative Medicine and Cellular Longevity, 2022*, 9299574.

Ichijo, H., Nishida, E., Irie, K., Dijke, P. T., Saitoh, M., Moriguchi, T., Takagi, M., Matsumoto, K., Miyazono, K., & Gotoh, Y. (1997). Induction of apoptosis by ASK1, a mammalian

MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science, 275*(5296), 90–94.

Jackson, C.C., Rupasinghe, H.P.V., Ayanbadejo, T., Martos, P. and Schooley, J. (2024). Assessment of Ontario grown ginseng for nutritional quqlity and food safety. *Acta Hortic*. 629, 115-121

Jin, Y., Cui, R., Zhao, L., Fan, J., & Li, B. (2019). Mechanisms of Panax ginseng action as an antidepressant. *Cell Proliferation, 52*(6), e12696.

Jung, J. H., Kang, I. G., Kim, D. Y., Hwang, Y. J., & Kim, S. T. (2013). The effect of Korean red ginseng on allergic inflammation in a murine model of allergic rhinitis. *Journal of Ginseng Research, 37*(2), 167–175.

Jung, M. Y., Jeon, B. S., & Bock, J. Y. (2002). Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (Panax ginseng C.A. Meyer). *Food Chemistry, 79*(1), 105–111.

Kaspersen, M. D., Bungum, M., & Fedder, J. (2013). Sperm DNA fragmentation in men with testicular cancer. *Andrology*, 1(5), 816–822.

Kang, S., & Min, H. (2012). Ginseng, the “immunity boost”: The effects of Panax ginseng on immune system. *Journal of Ginseng Research, 36*(4), 354–368.

Kang, S. Y., Schini-Kerth, V. B., & Kim, N. D. (1995). Ginsenosides of the protopanaxatriol group cause endothelium-dependent relaxation in the rat aorta. *Life Sciences, 56*(18), 1577–1586.

Khan, M. A., Ali, M. F., and Farhan, A. (2022). Cadmium-induced reproductive toxicity: Mechanisms and mitigation strategies. Reproductive Toxicology, 9(5):30-45.

Liu, J., Wang, X., and Li, Y. (2017). Cadmium exposure and body weight changes in rats. Environmental Toxicology, 32(1):141-148.

Kim, R., Son, S.-R., Lee, N.-K., Kim, J.-Y., An, G., Choi, J.-H., & Jang, D. S. (2022). Cytotoxic Properties of C_{17} Polyacetylenes from the Fresh Roots of *Panax ginseng* on Human Epithelial Ovarian Cancer Cells. *Molecules*, *27*(20), 7027

Kim, D. H. (2012). Chemical diversity of Panax ginseng, Panax quinquefolium, and Panax notoginseng. *Journal of Ginseng Research, 36*(1), 1–15.

Kim, J. H., Yi, Y. S., Kim, M. Y., & Cho, J. Y. (2017). Role of ginsenosides, the main active components of Panax ginseng, in inflammatory responses and diseases. *Journal of Ginseng Research, 41*(4), 435–443.

Kim, J. S. (2016). Investigation of phenolic, flavonoid, and vitamin contents in different parts of Korean ginseng (Panax ginseng C.A. Meyer). *Preventive Nutrition and Food Science, 21*(3), 263–270.

Knispel, N., Ostrozhenkova, E., Schramek, N., Huber, C., Peña-Rodríguez, L. M., Bonfill, M., Palazón, J., Wischmann, G., Cusidó, R. M., & Eisenreich, W. (2013). Biosynthesis of panaxynol and panaxydol in Panax ginseng. *Molecules, 18*(7), 7686–7698.

Kiran, R., Bharti, R., & Sharma, R. (2022). Effect of heavy metals: An overview. *Materials Today: Proceedings, 51*(1), 880–885.

Kitagawa, I., Taniyama, T., Shibuya, H., Noda, T., & Yoshikawa, M. (1987). Chemical studies on crude drug processing. V. On the constituents of ginseng radix rubra (2). *Yakugaku Zasshi, 107*(7), 495–505.

Kitagawa, I., Yoshikawa, M., Yoshihara, M., Hayashi, T., & Taniyama, T. (1983). Chemical studies of crude drugs (1). Constituents of ginseng radix rubra. *Yakugaku Zasshi, 103*(7), 612–622.

Laskey, J. W., & Phelps, P. V. (1991). Effect of cadmium and other metal cations on in vitro Leydig cell testosterone production. *Toxicology and Applied Pharmacology, 108*(2), 296–306.

Lee, J. H., Choi, K. H., Sohn, E. H., & Jang, K. H. (2013). Quality characteristics and ginsenosides composition of ginseng-yakju according to the particle size of ginseng powder. *Preventive Nutrition and Food Science, 18*(4), 234–241.

Lee, S. M., Bae, B., Park, H., Ahn, N., Cho, B., Cho, Y., & Kwak, Y. (2015). Characterization of Korean red ginseng (Panax ginseng Meyer): History, preparation method, and chemical composition. *Journal of Ginseng Research, 39*(4), 384–391.

Liu, C. X., & Xiao, P. G. (1992). Recent advances on ginseng research in China. *Journal of Ethnopharmacology, 36*(1), 27–38.

Liu, H., Lu, X., Hu, Y., & Fan, X. (2020). Chemical constituents of Panax ginseng and Panax notoginseng explain why they differ in therapeutic efficacy. *Pharmacological Research, 161*, 105263.

Liu, J., Qu, W., & Kadiiska, M. B. (2009). Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicology and Applied Pharmacology, 238*(3), 209–214.

Liu, Z., Wen, X., Wang, C. Z., Li, W., Huang, W. H., Xia, J., Ruan, C. C., & Yuan, C. S. (2020). Remarkable impact of amino acids on ginsenoside transformation from fresh ginseng to red ginseng. *Journal of Ginseng Research, 44*(3), 424–434.

Mishra, J. N., & Verma, N. K. (2017). An overview on Panax ginseng. *International Journal of Pharma and Chemical Research, 3*(3), 516–522.

Morshed, M. N., Ahn, J. C., Mathiyalagan, R., Rupa, E. J., Akter, R., Karim, M. R., Jung, D. H., Yang, D. U., Yang, D. C., & Jung, S. K. (2023). Antioxidant activity of Panax ginseng to regulate ROS in various chronic diseases. *Applied Sciences, 13*(5), 2893.

Motawee, M. E., Damanhory, A. A., Sakr, H., Khalifa, M. M., Atia, T., Elfiky, M. M., Maher, M., & Sakr, H. I. (2022). An electron microscopic and biochemical study of the potential protective effect of ginger against cadmium-induced testicular pathology in rats. *Frontiers in Physiology, 13*, 996020.

Niknafs, B., Salehnia, M. and Kamkar, M. (2015). Induction and determination of apoptotic and necrotic cell death by cadmium chloride in testis tissue of mouse. *Journal of Reproduction and Infertility*, 16(1): 24-29.

Nocerino, E., Amato, M., & Izzo, A. A. (2020). The aphrodisiac and adaptogenic properties of ginseng. *Fitoterapia, 71*(1), 1–5.

Park, S. J., Lee, J. R., Jo, M. J., Park, S. M., Ku, S. K., & Kim, S. C. (2013). Protective effects of Korean red ginseng extract on cadmium-induced hepatic toxicity in rats. *Journal of Ginseng Research, 37*(1), 37–44.

Patra, R. C., Rautray, A. K., & Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Veterinary Medicine International, 2011*, 457327.

Phytochemistry Reviews. (2005). Biological activities and chemistry of saponins from Panax ginseng C. A. Meyer. *Phytochemistry Reviews, 4*(3), 159–175.

Shojaeepour, S., Dabiri, S., Dabiri, B., Imani, M., Abadi, M. F. S., & Hashemi, F. (2021). Histopathological findings of testicular tissue following cadmium toxicity in rats. *Iranian Journal of Pathology, 16*(4), 348–355.

Shojaeepour, S., Sharififar, F., Haghpanah, T., Iranpour, M., Imani, M., & Dabiri, S. (2021). Panax ginseng ameliorates toxic effects of cadmium on germ cell apoptosis, sperm quality, and oxidative stress in male Wistar rats. *Toxin Reviews, 41*(2), 389–401.

Shibata, S., Ando, T., Tanaka, O., Meguro, Y., Sôma, K., & Iida, Y. (1965). Saponins and sapogenins of Panax ginseng C.A. Meyer and some other Panax spp. *Yakugaku Zasshi, 85*(7), 753–755.

Shibata, S., Tanaka, O., Nagai, M., & Ishii, T. (1963). Studies on the constituents of Japanese and Chinese crude drugs. XII. Panaxadiol, a sapogenin of ginseng roots. *Chemical and Pharmaceutical Bulletin, 11*(7), 762–765.

Shibata, S., Tanaka, O., Soma, K., Ando, T., Iida, Y., & Nakamura, H. (1965). Studies on saponins and sapogenins of ginseng. The structure of panaxatriol. *Tetrahedron Letters, 6*(42), 207–213.

Siu, E. R., Mruk, D. D., Porto, C. S., & Cheng, C. Y. (2009). Cadmium-induced testicular injury. *Toxicology and Applied Pharmacology, 238*(3), 240–249.

Suzuki, A., Matsuura, D., Kanatani, H., Yano, S., Tsunakawa, M., Matsuyama, S., & Shigemori, H. (2017). Inhibitory effects of polyacetylene compounds from Panax ginseng on neurotrophin receptor-mediated hair growth. *Biological & Pharmaceutical Bulletin, 40*(10), 1784–1788.

Sharma, P., Huq, A. U., & Singh, R. (2014). Cyto-protective role of Panax ginseng against oxidative stress induced by cadmium in male rats. Journal of Environmental Pathology, Toxicology and Oncology, 33(2), 109–123.

Thompson, J., & Bannigan, J. (2008). Cadmium: Toxic effects on the reproductive system and the embryo. *Reproductive Toxicology, 25*(3), 304–315.

Tu, Y. (2016). Artemisinin: A gift from traditional Chinese medicine to the world (Nobel Lecture). *Angewandte Chemie International Edition, 55*(35), 10210–10226.

Van Kampen, J. M., Baranowski, D. B., Shaw, C. A., & Kay, D. G. (2014). Panax ginseng is neuroprotective in a novel progressive model of Parkinson's disease. *Experimental Gerontology, 50*, 95–105.

Wang, J., Li, S., Fan, Y., Chen, Y., Liu, D., & Cheng, H. (2012). Anti-fatigue activity of Panax ginseng extracts in mice. Journal of Ethnopharmacology, 139(2), 244–250.

Wang, Y., Sun, J., & Chen, B. (2010). Effects of Panax ginseng on sperm motility and testicular oxidative stress in cadmium-treated rats. Toxicology and Industrial Health, 26(1), 47–56.

Wang, H. P., Yang, X. B., Yang, X. W., Liu, J. X., Wang, Y. P., & Zhang, L. X. (2013). [Article in Chinese]. *Zhongguo Zhongyao Zazhi, 38*(17), 2807–2817.

Wang, Y., Zhang, Y.,and Xu, D. (2020). Effects of cadmium on body weight and testes weight in mice. *Journal of Environmental Science and Healt*, 55, 1-8.

Xu, D., Wang, Y.,and Zhang, Y. (2018). Cadmium-induced testicular toxicity in rats. Toxicology, 3(9):42-49.

Washida, D., & Kitanaka, S. (2003). Determination of polyacetylenes and ginsenosides in Panax species using high performance liquid chromatography. *Chemical and Pharmaceutical Bulletin, 51*(11), 1314–1317.

Xu, B., Chen, S., Luo, Y., Chen, Z., & Liu, L. (2020). Cadmium-induced testicular injury by disrupting blood-testis barrier and promoting inflammation in rats. *Environmental Toxicology, 35*(3), 443–452.

Xu, X. F., Gao, Y., Xu, S. Y., Liu, H., Xue, X., Zhang, Y., & Li, X. R. (2018). Remarkable impact of steam temperature on ginsenosides transformation from fresh ginseng to red ginseng. *Journal of Ginseng Research, 42*(3), 277–287.

Xu, J., Jia, L., & Zhao, Q. Y. (2011). Ginseng ingredients and ginseng product quality evaluation. *Drug Evaluation Research, 34*(3), 199–203.

Yang, Y., Ju, Z., Yang, Y., Zhang, Y., Yang, L., & Wang, Z. (2021). Phytochemical analysis of Panax species: A review. *Journal of Ginseng Research, 45*(1), 1–21.

Yang, Y., Ju, Z., Yang, Y., Zhang, H., Sun, Z., & Zhang, Y. (2014). Protective effects of ginsenoside Rg1 on cadmium-induced testicular toxicity in mice. *Biological Trace Element Research, 162*(1–3), 207–214.

Yokozawa, T., Kobayashi, T., Oura, H., & Kawashima, Y. (1987). Studies on the mechanism of the hypoglycemic activity of ginsenoside-Rb2 in streptozotocin-diabetic rats (II). *Chemical and Pharmaceutical Bulletin, 35*(9), 3570–3575.

Yoo, H. H., Park, J. H., Kwon, S. W., & Kim, Y. C. (2002). Effects of ginsenosides on the hepatic drug-metabolizing enzyme system in mice treated with carbon tetrachloride. *Biological & Pharmaceutical Bulletin, 25*(4), 457–460.

Zhang, J., Wang, Y., Dong, Y., & Yang, X. (2017). Protective effect of Panax ginseng on cadmium-induced testicular toxicity in rats. *Journal of Ethnopharmacology, 206*, 200–210.

Zhai, Q., Narbad, A., & Chen, W. (2015). Dietary strategies for the treatment of cadmium and lead toxicity. *Nutrients*, 7(1), 552–571.

Zhou, J., Li, X., He, J., & Li, X. (2019). Advances in research on the pharmacological effects of ginsenosides. *Chinese Medicine, 14*, 42.

APPENDIX I

The instrument used for this research is as follows:

Animal House: during the time of feeding.

Feeding plate

Feeding water container

Feed (pellets)

ISOL disinfectant

Digital thermometer

Plastic cage

Weighing balance

Indian ink

For Sacrificing

Hand gloves

Dissecting instruments

Cotton wool

Chloroform

Sterile universal container

Weighing balance

Formalin

Histology Laboratory

Scrape blade

Spatula

Block holder

Automatic tissue processor

Tissue basket

L-shaped mould

Rotary microtome

Floatation bath

Hot plate

Metal pencil

Slides and cover slip

Stain (Haematoxylin and eosin)

Binocular microscope

Dibutylphthalate polysterene xylene (DPX),

Xylene, alcohol and water

APPENDIX II

PROCEDURE FOR TISSUE PROCESSING

TISSUE (testis) processing using manual method. Sequences for manual tissue processing were as follows:

Harvesting Tissue: The required tissues (testis) were harvested from the animals and immediately put in a fixative. All parts of the required tissue that showed obvious microscopic changes were essentially selected for sampling. Tissues were cut into thin slices of 3mm by size.

Selection of Tissue: The testis (oval-shaped) and were colored. They were pinkish to light brown in the scrotum. It is part of the male reproductive system. It is located outside the body, suspended in the scrotal sac, and is connected to the spermatic cord, lying between the epididymis and the start of the vas deferens.

Fixation: The fixation used was 10% Bouin fluid (prepared using a saturated picric acid solution by dissolving 13.6 g picric acid in 100 mL distilled water, mix 75 mL saturated picric acid solution with 25 mL 40% formaldehyde solution, add 5 mL glacial acetic acid), was carried out for 24 hours to ensure proper fixing of the testicular tissues.

Dehydration: Tissues was dehydrated by using increasing strength of alcohol from 70%, 90% and absolute alcohol. All at varying interval of time to ensure proper dehydration. The volume of alcohol used was 50 - 100 times of that of tissues.

70% alcohol	……	2hours
90% alcohol	……	2hours
95% alcohol	……	2hours
Absolute alcohol I	……	2hours
Absolute alcohol II	……	2hours
Absolute alcohol III	……	2hours

Clearing: Tissues was cleared by passing the tissue through two changes of xylene.

Xylene I	……	90 minutes

Xylene II …… 90 minutes

Impregnation with Wax: This was carried out at the melting point temperature of paraffin wax; volume of wax was about 25 - 30 times the volume of tissues. The duration of impregnation lasted for two hours each in two changes of wax to ensure proper impregnation.

Paraffin wax I …… 2hours

Paraffin wax II …… 2hours

Embedding: Impregnated tissues were orientated in tissue molds, fresh molten wax was poured in it and allowed to solidify in the cooling chamber. Afterwards they were timed and sectioned.

PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

Staining of Processed Tissues Principle: Hematoxylin is a basic dye and thus has affinity for the acidic part of the cellular component which is the nucleus. Therefore, the nucleus stains blue while eosin on the other hand is an acidic dye thus has affinity for the basic component of the cells which is the cytoplasm therefore it stains it pink which is the color of the dye. This staining procedure was facilitated with a mordant that linked the stain to the tissue and a differentiator (acid alcohol) that differentiated the nuclear stain from cytoplasmic stain.

The sections were dewaxed in two changes of xylene for 2 minutes each.

Sections were taken through descending grades of alcohol. From absolute alcohol for 2 minutes, to 90% alcohol for 1 minutes, 70% alcohol for 1 minutes.

The slides were washed in running tap water for one minute.

Tissue sections were stained in hematoxylin for 10 minutes

The tissue sections rinsed in distilled water for 30 seconds.

Sections were differentiated in 1% acid alcohol for 15 seconds

Sections were rinsed in distilled water for 5 minutes.

Sections were counter stained with 1% eosin for 5 minutes.

Sections were washed in running tap water for 30 seconds.

Sections were dehydrated by passing through ascending grades of alcohol (70%, 90%, and 100%) for 1 minute each.

Sections were cleared in two changes of xylene for 2 minutes each.

Sections were mounted with DPX and viewed microscopically using objective lenses.

After which, slides were stained according to Hematoxylin and Eosin method

APPENDIX III

MINISTRY OF AGRICULTURE AND FOOD SECURITY,
ANIMAL ETHICS COMMITTEE (MAFSAEC)

CERTIFICATE
OF ETHICAL APPROVAL

This is to certify that

EHIOZEE EDNA ISOKEN

Has been given MAFSAEC Approval for the Animal Component of the research titled:

HISTOPATHOLOGICAL EFFECTS OF PANNAX GINSENG ON CADMIUM INDUCED TESTICULAR TOXICITY IN WISTAR ALBINO RATS.

In accordance with the Animal Disease Control Act, 2022.

Dr L.I Adebudo
Chairman MAFSAEC

Approval No.
MAFSAEC: 025-11/12/0049

Date Of Approval
12th November, 2025

(This Approval is only valid for this study)


SPECIMEN AFTER CUT-UP


LEICA RM2235