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To Investigate the Potential Mechanisms By Which A Combination of Zinc, Fructose, And Pumpkin Seeds Affects Infertility-Related Diseases

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ABSTRACT

Infertility, particularly male infertility, is a growing global concern with declining sperm quality being a major contributing factor. The present study evaluated the effects of combined supplementation of zinc, fructose, and pumpkin seeds on reproductive function and systemic health in male Wistar rats. Twenty-four rats were divided into four groups: control, 100 mg/kg, 150 mg/kg, and 200 mg/kg, administered orally for eight weeks. Hematological, biochemical, hormonal, and semen parameters were assessed alongside histopathological examinations of vital organs. Results showed that 100 mg/kg supplementation improved reproductive health with enhanced germ cell development and stable systemic function. The 150 mg/kg group exhibited the most beneficial effects, including optimal hematological and biochemical stability, normal liver and kidney function, high-normal testosterone levels (7.2–7.6 ng/mL), and significantly improved semen parameters (sperm count 100–118 million/mL, motility 76–84%, progressive motility 54–68%, viability 86–92%, morphology 74–85%, semen volume ~2.0 mL). Histological findings confirmed well-preserved tissue integrity across multiple organs with vigorous spermatogenesis. Conversely, the 200 mg/kg group demonstrated peak semen parameters (sperm count 120–122 million/mL, motility 88–92%), but severe systemic toxicity including renal tubular necrosis, testicular degeneration, pancreatic and cardiac damage, and fibrosis in reproductive tissues. Overall, supplementation with zinc, fructose, and pumpkin seeds at moderate levels (100–150 mg/kg) enhanced male fertility, with 150 mg/kg identified as the most effective and safest dose, whereas supraphysiologic dosing (200 mg/kg) caused significant organ toxicity.

Keywords: *Male Reproductive Health, Westar Rats, Zinc-Fructose- Pumpkin Seeds, Dose-Dependent Effects, Testicular Histology, Sperm Parameters, Testosterone Levels.*

1. INTRODUCTION

Background on Male Infertility:

Male infertility is a significant global health concern, affecting approximately 10–15% of couples, with male factors contributing to nearly 50% of these cases (Agarwal et al., 2021; Kumar & Singh, 2015). The etiology of male infertility is multifactorial, encompassing genetic abnormalities, infections, varicocele, endocrine disorders, lifestyle factors (such as smoking, alcohol consumption, and obesity), and exposure to environmental pollutants (Esteves et al., 2021; Durairajanayagam et al., 2015). Among these, oxidative stress has emerged as a key mechanism impairing male reproductive function by disrupting the structural and functional integrity of spermatozoa (Aitken et al., 2014; Tremellen, 2008).

Oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms in the body. While physiological levels of ROS are essential for normal sperm capacitation and acrosome reaction, excessive ROS levels can lead to lipid peroxidation of the sperm membrane, DNA fragmentation, mitochondrial dysfunction, and apoptosis, ultimately resulting in reduced sperm motility, viability, and fertilization potential

(Sharma et al., 2019; Agarwal et al., 2006). In fact, oxidative stress is detected in up to 80% of infertile men, making it one of the most common pathological mechanisms associated with male subfertility (Agarwal et al., 2006; Henkel, 2011).

To counteract oxidative stress and restore sperm function, the role of antioxidants has been extensively studied. Antioxidants are molecules capable of neutralizing ROS and protecting cellular components from oxidative damage. A growing body of evidence supports the therapeutic potential of antioxidant supplementation in improving sperm parameters and overall reproductive outcomes in infertile males (Gharagozloo & Aitken, 2011; Showell et al., 2014). Among various antioxidants, natural dietary supplements—including vitamins (e.g., C, E), minerals (e.g., zinc, selenium), coenzymes (e.g., CoQ10), amino acids (e.g., L-carnitine), and phytochemicals (e.g., flavonoids, polyphenols)—have received considerable attention due to their efficacy, safety, and accessibility (Martínez-Soto et al., 2016; Ko et al., 2014; Majzoub & Agarwal, 2018).

Mitochondria play a pivotal role in sperm function and male fertility by acting as the powerhouse of the cell and regulating several essential cellular mechanisms. Primarily located in the midpiece of the spermatozoon, mitochondria generate adenosine triphosphate (ATP) through oxidative phosphorylation, supplying the energy necessary for sperm motility, capacitation, hyperactivation, and ultimately, successful fertilization (Amaral et al., 2013; Paoli et al., 2011). Efficient mitochondrial energy production enhances flagellar activity, which is essential for the progressive movement of sperm through the female reproductive tract (Ruiz-Pesini et al., 2007). Additionally, mitochondria are key regulators of reactive oxygen species (ROS), which, in low to moderate concentrations, support physiological processes like capacitation and the acrosome reaction (Agarwal et al., 2014). However, an imbalance leading to excessive ROS generation results in oxidative stress, causing damage to sperm DNA, lipids, and proteins, thereby reducing fertility potential (Sharma et al., 2021; Sakkas & Alvarez, 2010).

Antioxidants such as zinc and bioactive components found in pumpkin seeds have shown efficacy in mitigating oxidative stress. Zinc acts as a cofactor for several antioxidant enzymes, helps stabilize cell membranes, and protects against lipid peroxidation in sperm (Fallah et al., 2018; Wong et al., 2002). Mitochondria also regulate apoptosis by removing defective germ cells during spermatogenesis, thus ensuring the production of functionally competent sperm (Galluzzi et al., 2012). Moreover, mitochondrial control of intracellular calcium levels is essential for sperm hyperactivation—a vigorous motility pattern required to penetrate the zona pellucida of the oocyte (Darszon et al., 2006). Mitochondrial biogenesis, a process modulated by nutrients such as zinc and co-factors like coenzyme Q10, plays a significant role in enhancing both the quantity and quality of mitochondria, further improving sperm functionality (Martínez-Patiño et al., 2021; Nassan et al., 2018).

Zinc, fructose, and pumpkin seeds are among natural agents that demonstrate potential therapeutic value in the management of oxidative stress-induced male infertility. Zinc is a critical trace mineral for male reproductive function, contributing to spermatogenesis, sperm chromatin stability, and motility (Fallah et al., 2018; Colagar et al., 2009). Fructose serves as a principal energy substrate for sperm motility and is naturally secreted in seminal plasma, aiding in sustained flagellar motion (Mann, 1964; Hamamah & Gatti, 1998). Pumpkin seeds (*Cucurbita moschata*) are rich in polyunsaturated fatty acids, phytosterols, and antioxidant compounds such as vitamin E, zinc, and selenium, which collectively support reproductive health and protect against testicular damage and oxidative imbalance (Gossell-Williams et al., 2006; Khazaei et al., 2017).

The combination of zinc, fructose, and pumpkin seed extract, administered at varying doses, may offer a synergistic effect by enhancing mitochondrial function, reducing oxidative stress, and supporting sperm maturation and motility. This multifaceted approach holds promise in improving sperm parameters and overall male fertility outcomes.

The Role of Oxidative Stress in Male Infertility

Oxidative stress plays a pivotal role in the pathogenesis of male infertility and is widely recognized as one of its leading causes. Reactive oxygen species (ROS), which are naturally produced as byproducts of cellular metabolism, are involved in several physiological processes related to sperm function, including capacitation, hyperactivation, and acrosome reaction when maintained at low levels. However, an overproduction of ROS or a compromised antioxidant defense system results in oxidative stress, which can severely impair male reproductive potential (Agarwal et al., 2020; Aitken & Baker, 2006).

Spermatozoa are uniquely vulnerable to oxidative damage because of their high content of polyunsaturated fatty acids in the plasma membrane and their limited cytoplasmic space, which restricts the availability of antioxidant enzymes (Aitken RJ, Clarkson JS, 1987). Excessive ROS leads to lipid peroxidation, which compromises membrane fluidity and integrity, thus impairing motility and the ability of sperm to fertilize the oocyte (Kao SH et al., 2008). Furthermore, ROS can induce oxidative modifications in sperm DNA, leading to fragmentation, chromatin cross-linking, and apoptosis—all of which are associated with reduced fertilization rates and increased risk of miscarriage (Muratori et al., 2015; Henkel, 2011).

Oxidative stress also interferes with mitochondrial function, causing a decline in ATP production and further diminishing sperm motility (Amaral et al., 2013). Moreover, oxidative insults can impair the acrosome reaction—a necessary step for oocyte penetration—thereby reducing fertilization potential (Agarwal et al., 2017). Several clinical studies have shown significantly elevated levels of ROS in the semen of infertile men compared to fertile controls, often accompanied by reduced total antioxidant capacity (Huang C et al., 2019; Tremellen, 2008).

Given this, there is increasing interest in antioxidant-based therapies to mitigate oxidative stress and improve fertility outcomes. Nutritional antioxidants such as vitamin C, vitamin E, zinc, selenium, Coenzyme Q10, L-carnitine, and plant-based polyphenols have shown promise in reducing ROS levels and improving sperm parameters (Sharma R et al., 2019; Showell MG et al., 2020). Both human and animal studies have demonstrated improvements in sperm motility, morphology, and DNA integrity following antioxidant supplementation (Gharagozloo & Aitken, 2011; Banihani, 2019).

Thus, targeting oxidative stress represents a valuable therapeutic strategy for managing idiopathic male infertility and enhancing reproductive outcomes.

Zinc and Its Role in Reproductive Health

Zinc is a vital trace element essential for numerous physiological functions, particularly those associated with the male reproductive system. It plays a crucial role in **spermatogenesis, DNA synthesis, cell division, enzymatic activity, and hormonal regulation**, especially testosterone metabolism (Prasad AS, 2013; Wessels et al., 2017). Adequate zinc levels are necessary for testicular development, the maintenance of germinal epithelium, and the production of viable, motile spermatozoa (Fallah A et al., 2018).

Zinc deficiency has been correlated with a spectrum of reproductive dysfunctions, including **hypogonadism, reduced testosterone levels, low sperm count, poor sperm motility, and abnormal morphology** (Colagar AH et al., 2009; Kumar N & Singh AK, 2015). Zinc is intricately involved in **chromatin stability**, as it binds to protamines—proteins that compact and protect sperm DNA during spermiogenesis, thus ensuring sperm genomic integrity (Colagar AH et al., 2009).

In addition, zinc contributes to **antioxidant defense mechanisms** by serving as a cofactor for enzymes such as superoxide dismutase (SOD), which neutralize reactive oxygen species (ROS) and protect sperm cells from oxidative stress—a major contributor to male infertility (Khan S et al., 2020; Liu K et al., 2018). Excessive ROS can damage the sperm plasma membrane, DNA, and mitochondria, reducing fertilizing potential.

Several studies have demonstrated that **zinc supplementation can significantly enhance semen parameters**, including sperm concentration, motility, and morphology. For instance, Omu et al. (2008) reported improvements in sperm function and hormonal profiles in infertile men following oral zinc therapy. Similarly, Fallah et al. (2018) showed that zinc supplementation in men with idiopathic infertility improved testosterone levels and various sperm characteristics.

Beyond semen quality, zinc may also modulate the hypothalamic-pituitary-gonadal (HPG) axis, stimulating luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release, both of which are crucial for testicular function (Wang L et al., 2016). Its **anti-inflammatory and immunomodulatory properties** further contribute to reproductive protection by mitigating testicular inflammation and cellular apoptosis (Yamaguchi M, 2006).

In the context of this study, zinc is anticipated to exert **dose-dependent protective and enhancing effects on male reproductive health** through both direct antioxidant activity and indirect hormonal modulation. The graded doses of zinc (100 mg/kg, 150 mg/kg, and 200 mg/kg) will be evaluated for their efficacy in improving sperm quality, fertility indices, and testicular histology in albino Wistar rats, particularly under stress or toxicological insult.

Fructose as an Energy Source for Sperm

Fructose, a naturally occurring monosaccharide, plays a pivotal role as an energy substrate in the male reproductive system, particularly for spermatozoa. It is predominantly secreted by the seminal vesicles and is found in high concentrations within the seminal plasma (Rodríguez-Gil & Miró, 2012). The primary function of fructose in this context is to fuel sperm motility through glycolysis, a metabolic pathway that generates adenosine triphosphate (ATP), the essential energy currency of cells (Mortimer, 1997; Mann, 1964). Sperm motility is vital for successful fertilization, as motile sperm must travel significant distances through the female reproductive tract to reach and penetrate the oocyte. This journey demands substantial and sustained energy production. Fructose, due to its rapid metabolism via glycolytic pathways, provides an immediate and efficient energy source, thereby supporting progressive motility and sperm viability (Nassar et al., 1999; Ford, 2006). A number of studies have demonstrated a strong correlation between seminal fructose levels and sperm motility parameters. Low seminal fructose concentration has been associated with asthenozoospermia, a condition marked by impaired sperm motility and a common contributor to male infertility (Carrell et al., 2003; Henkel et al., 2005). This suggests that a reduced supply of metabolic energy substrates, such as fructose, may compromise sperm functionality. Beyond energy production, fructose is also implicated in the regulation of capacitation and the acrosome reaction, critical events that enable sperm to fertilize the ovum. The acrosome reaction involves the release of enzymes that digest the zona pellucida of the egg, and energy availability is believed to influence the efficiency and timing of this process (Lishko et al., 2012; Gibb et al., 2014). Moreover, dietary fructose has been studied for its potential role in enhancing sperm parameters when administered under controlled conditions. Supplementation of fructose has been shown to increase sperm count, motility, and membrane integrity in various experimental models, indicating a positive effect on reproductive function (Mostafa et al., 2007; Amaral et al., 2013). In this study, the effects of fructose supplementation at graded doses (100 mg/kg, 150 mg/kg, and 200 mg/kg) are being evaluated in the context of improving sperm motility and overall reproductive health. When combined with zinc and pumpkin seeds (*Cucurbita moschata*), both known for their reproductive and antioxidant benefits, this intervention aims to explore potential synergistic effects in ameliorating infertility-related parameters. The hypothesis is that this combination may enhance sperm function by not only providing metabolic energy but also by mitigating oxidative stress and promoting testicular health.

Pumpkin Seeds: A Rich Source of Antioxidants

Pumpkin seeds (*Cucurbita pepo* and *Cucurbita moschata*) have long been recognized for their medicinal properties, particularly their positive effects on male reproductive health. These seeds are a rich natural source of antioxidants, including vitamin E, carotenoids, selenium, and polyphenols, which collectively play a vital role in neutralizing reactive oxygen species (ROS) and mitigating oxidative damage to sperm cells (Gossell-Williams et al., 2006; Nkosi et al., 2005). Oxidative stress is a major contributor to male infertility, impairing sperm function through lipid peroxidation and DNA fragmentation (Agarwal et al., 2006). The antioxidant profile of pumpkin seeds supports their potential to protect spermatozoa from such damage. Moreover, pumpkin seeds are a notable dietary source of zinc, an essential trace element that plays a critical role in spermatogenesis, testosterone synthesis, and antioxidant defense systems (Fallah et al., 2018; Omu et al., 1998). Tsai et al. (2016) demonstrated that pumpkin seed extract significantly improved sperm parameters—including count, motility, and morphology—in rats with induced infertility, primarily by reducing oxidative stress and lipid peroxidation. These findings align with earlier research by Gossell-Williams et al. (2006), who reported that administration of pumpkin seed oil had a protective effect on prostate and testicular tissues due to its anti-inflammatory and antioxidant activity. Additionally, pumpkin seeds are a rich source of essential fatty acids such as linoleic and oleic acid, which contribute to the structural integrity of sperm membranes and help reduce inflammation (Cheikh-Rouhou et al., 2007). These fatty acids not only protect against oxidative stress but also enhance the fluidity and function of the sperm cell membrane, a crucial factor for fertilization capability. Studies have also indicated immunomodulatory and hormone-regulating effects of pumpkin seed supplementation, supporting its use in managing conditions like benign prostatic hyperplasia and improving overall testicular health (Glew et al., 2006; Carotenuto et al., 2022). Given its broad spectrum of bioactive compounds, pumpkin seed supplementation appears to be a promising strategy for improving male fertility. Therefore, the present study will evaluate the dose-dependent effects of pumpkin seed extract at concentrations of 100 mg/kg, 150 mg/kg, and 200 mg/kg on sperm function, oxidative stress markers, and reproductive hormone levels in Wistar albino rats.

Synergistic Effects of Zinc, Fructose, and Pumpkin Seeds

The rationale behind combining **zinc**, **fructose**, and **pumpkin seeds (Cucurbita moschata)** in addressing male infertility lies in their **complementary physiological roles** in enhancing sperm function, hormonal balance, and antioxidant defense. Individually, each component contributes significantly to reproductive health, but their **combination may yield synergistic effects** that outperform monotherapies by simultaneously targeting multiple pathways involved in spermatogenesis and sperm function. **Zinc** plays a critical role in **spermatogenesis**, **testosterone synthesis**, and **sperm motility**, serving as a structural component of numerous metalloenzymes and transcription factors involved in DNA replication and repair (Fallah et al., 2018). It also acts as an **antioxidant**, reducing reactive oxygen species (ROS) in seminal plasma (Agarwal et al., 2008). Zinc deficiency has been associated with reduced sperm count, motility, and altered morphology (Kothari et al., 2010).

Fructose is a principal **energy substrate** for spermatozoa. It is secreted by seminal vesicles and utilized by sperm cells to fuel **flagellar motility** and prolong **survival** during their journey through the female reproductive tract (Laleye et al., 2019). Sperm motility is energy-dependent, and insufficient fructose availability has been linked with asthenozoospermia and male subfertility (Amelar & Dubin, 1970).

Pumpkin seeds (Cucurbita spp.) are rich in **phytonutrients**, **essential fatty acids**, **magnesium**, and **antioxidants** such as **vitamin E**, **selenium**, and **carotenoids**, which combat lipid peroxidation and ROS-induced sperm damage (Gossell-Williams et al., 2006). They also contain **phytosterols** that may contribute to improved prostate and testicular health and enhanced testosterone levels (Nkosi et al., 2005). Several **studies suggest that combination therapies** involving antioxidants, trace elements, and energy substrates may be **more efficacious** than single agents in enhancing male fertility. Gossell-Williams et al. (2006) demonstrated that pumpkin seed oil in combination with vitamin E led to significant improvements in sperm count and motility in rats. A study by Hamidia et al. (2009) also supported the **additive effects** of antioxidant combinations in protecting sperm from oxidative damage. Likewise, **co-administration of zinc with other antioxidants** has been shown to potentiate its protective effects on testicular tissue and sperm DNA (Ebisch et al., 2007; Omu et al., 2008). These findings align with the hypothesis that the **zinc–fructose–pumpkin seed triad** may **synergistically** optimize reproductive outcomes by supporting **hormonal regulation**, **membrane stability**, **mitochondrial function**, and **ROS scavenging** simultaneously. In the present study, three different **dose combinations (100 mg/kg, 150 mg/kg, and 200 mg/kg)** will be evaluated to assess their **dose-dependent efficacy** in improving sperm parameters, antioxidant enzyme activity (e.g., SOD, CAT, GPx), and reproductive hormone levels (testosterone, LH, FSH). This dose-ranging approach is designed to determine whether **higher concentrations amplify synergistic effects** or reach a plateau of therapeutic benefit. Overall, the inclusion of zinc, fructose, and pumpkin seeds in a combined regimen is hypothesized to offer **multifactorial support** for the male reproductive system, improving fertility through antioxidant protection, energy provision, and hormonal modulation.

Current Gaps in Research and Study Objectives

Despite the growing body of evidence supporting the use of antioxidants in the treatment of male infertility, there is still a need for more research on combination therapies. Most studies have focused on the effects of individual antioxidants, and few have explored the potential synergistic effects of combining different agents. Furthermore, there is limited research on the dose-dependent effects of these agents, particularly in relation to their impact on sperm function and oxidative stress markers.

This study aims to address these gaps by investigating the potential mechanisms by which a combination of zinc, fructose, and pumpkin seeds affects infertility-related diseases. The study will assess the effects of different doses (100 mg/kg, 150 mg/kg, and 200 mg/kg) on sperm parameters, oxidative stress markers, and reproductive hormone levels in an experimental model. The findings of this study could provide valuable insights into the potential benefits of combination therapies for treating male infertility.

Study Objectives and Hypotheses:

The primary objective of this study is to investigate the possible mechanisms by which the combination of pumpkin seeds, zinc, and fructose influences infertility-related diseases. Specifically, this study aims to:

1. Evaluate the effects of different doses (100 mg, 150 mg, and 200 mg/kg of body weight) of the combination on sperm parameters, including count, motility, and morphology in male albino Wistar rats.

2. Assess the impact of the combination on serum testosterone levels.

3. Examine histopathological changes in the reproductive organs following supplementation with the combination.

4. Identify potential mechanisms underlying the observed effects, focusing on antioxidant activity, hormonal regulation, and energy provision.

Hypotheses:

1. The combination of pumpkin seeds, zinc, and fructose will improve sperm count, motility, and morphology compared to control groups.

2. The combination will lead to increased serum testosterone levels in treated groups.

3. Histopathological examination will reveal improved structural integrity and function of reproductive organs in treated groups.

4. The observed effects will be dose-dependent, with the 200 mg/kg dose potentially showing the most pronounced benefits.

2. MATERIALS AND METHODS

Study Design

This study aimed to assess the impact of a combined supplementation of zinc, fructose, and pumpkin seeds on sperm count in male Wistar rats. A total of 24 male albino Wistar rats, aged between 8 and 10 weeks and weighing 180–220 grams, were used. The animals were maintained under standard laboratory settings, including a 12-hour light/dark cycle, a controlled ambient temperature of 22 ± 2°C, and unrestricted access to standard rodent feed and water. The study protocol adhered to ethical standards for animal experimentation and received approval from the Institutional Animal Care and Use Committee (IACUC).

Experimental Animal Model

The experimental model involved healthy male Wistar rats of similar age and body weight range (8–10 weeks; 180–220 g). The animals were housed under consistent environmental conditions with regulated temperature and lighting, and had free access to food and water. The rats were randomly allocated into four groups (n=6 per group) to evaluate the effects of the nutrient combination on sperm parameters. The **Control Group** received a vehicle solution without active ingredients. The remaining groups were given the combination treatment at varying dosages: **100 mg/kg**, **150 mg/kg**, and **200 mg/kg** of body weight, respectively. All treatments were administered orally using a gavage once daily for a total duration of eight weeks.

Dose Preparation and Administration

The preparation and administration of doses were carried out with precision, based on the intended total dosage for each experimental treatment group. Three specific dosage levels were selected: 100 mg, 150 mg, and 200 mg, each consisting of a combination of zinc, fructose, and pumpkin seeds in carefully measured proportions. For the 100 mg dose, the formulation comprised 10 mg of zinc, 15 mg of fructose, and 75 mg of pumpkin seed powder. The 150 mg dose included 15 mg of zinc, 25 mg of fructose, and 110 mg of pumpkin seed powder. The highest dose, 200 mg, consisted of 20 mg of zinc, 30 mg of fructose, and 150 mg of pumpkin seed powder.

These formulations were strategically designed to reflect common dietary supplement compositions. Zinc accounted for approximately 15–20% of the total dose, fructose contributed around 30–40%, and pumpkin seed powder made up about 40–55%. This proportioning aimed to ensure a nutritionally balanced blend with the potential to enhance male reproductive health through synergistic effects of the components. The consistent methodology in dose preparation helped maintain uniformity across the study groups.

Future evaluations focusing on the bioavailability, metabolism, and absorption efficiency of each component in Albino Wistar rats will be essential. Such assessments would not only improve the accuracy of the dosing protocol but also enhance the reliability and translational potential of the study's findings regarding male reproductive function.

Blood Collection for Hormonal Evaluation

Hormonal assessments were carried out using blood samples collected through standard techniques such as tail vein extraction or cardiac puncture. Approximately 0.5 to 1 mL of blood was drawn from each animal. To isolate serum, the samples were centrifuged at 3,000 to 5,000 revolutions per minute for 10 minutes at a temperature of 4°C. The resulting serum was either stored at −20°C for short-term use or preserved at −80°C for extended storage. Hormonal profiling involved the quantification of key reproductive hormones, including testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin. These concentrations were determined using either enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) techniques.

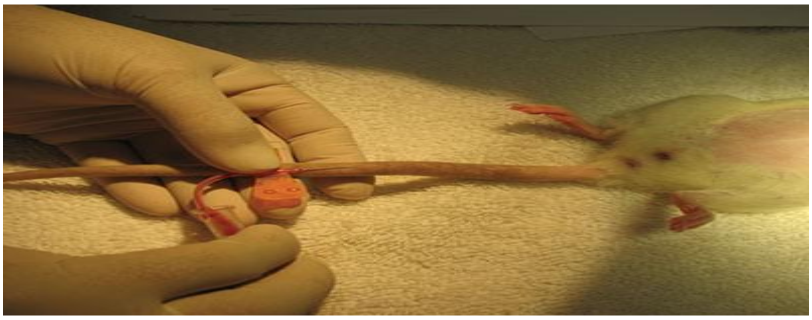


Figure 1: To investigate the potential mechanisms by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.-Blood collection from the tail of a rat.

Collection of Testicular and Epididymal Tissues for Histological Examination

Following euthanasia—typically performed using a CO_2 overdose or a high dose of anesthesia—the testes and epididymides were carefully excised through dissection. The collected tissues were immediately immersed in 10% formalin and fixed for 24 to 48 hours to maintain their anatomical structure for subsequent histological evaluation. After fixation, the samples were transferred into a 70% ethanol solution for long-term preservation prior to further processing. This procedure is essential for maintaining tissue morphology, enabling precise microscopic analysis and evaluation of structural integrity.

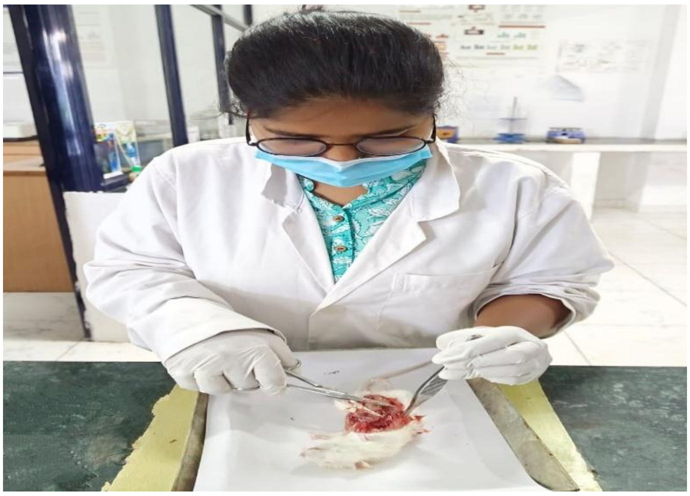


Figure 2: To investigate the potential mechanisms by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.- collection of testicular and epididymal tissues for histological examination.

Figure 3. - To investigate the potential mechanisms by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.- Organs retrieval.

Figure 4 - To investigate the potential mechanisms by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.-Testes retrieval.

Tissue Processing

Following fixation, tissue samples underwent a dehydration procedure involving a graded ethanol series, starting at 70% and progressing to 100%, to effectively remove water content. Subsequently, the tissues were cleared in xylene to facilitate optimal infiltration of the embedding medium. The samples were then embedded in paraffin wax, providing structural stability for thin sectioning. Using a microtome, sections approximately 5–7 µm thick were precisely cut for microscopic analysis. To visualize tissue morphology, standard staining techniques such as Hematoxylin and Eosin (H&E) staining were employed, enabling clear observation of cellular features and overall tissue architecture.

Figure 5: To investigate the potential mechanisms by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.-tissue sectioning.

Sperm Count

Sperm count, a critical indicator of male fertility, was assessed using established, standardized techniques to ensure measurement accuracy. Sperm samples were obtained from the epididymal duct by carefully excising the tissue and flushing it with a buffer solution like phosphate-buffered saline (PBS) under microscopic guidance. The retrieved spermatozoa were then quantified using an automated cell counter, allowing for precise and reproducible determination of sperm concentration. Consistent sampling techniques, careful handling of specimens, and proper storage practices were essential to maintain the reliability and validity of the sperm count and associated hormonal evaluations.

3. RESULTS

Across the groups, the control rats largely retained normal hematological, biochemical, hormonal, and reproductive profiles, though minor histological stress and a slight decline in sperm motility reflected natural physiological variations. Supplementation at 100 mg/kg with zinc, fructose, and pumpkin seeds led to marked improvements in systemic and reproductive health. Blood and biochemical markers remained steady, hormones were balanced, and semen parameters (sperm count ~75–76 million/mL, motility 65–68%, progressive motility 50–54%, and morphology 70–74%) surpassed those of the controls. Histological analysis revealed recovery in multiple organs and enhanced germ cell activity. The 150 mg/kg group showed the most favorable results, with strong hematological stability, intact liver and kidney function, elevated but normal testosterone levels (7.2–7.6 ng/mL), balanced gonadotropins, and superior semen characteristics (sperm count 100–118 million/mL, motility 76–84%, progressive motility 54–68%, viability 86–92%, morphology 74–85%, semen volume ~2.0 mL). Tissue studies confirmed preserved hepatic, renal, cardiac, pulmonary, and reproductive structures, along with active spermatogenesis and Leydig cell function. Conversely, the 200 mg/kg group displayed excessive hematological and hormonal stimulation, coupled with peak semen parameters (sperm count 120–122 million/mL, motility 88–92%, progressive motility 74–75%, viability 94–96%, morphology 82–90%). However, histopathology indicated extensive toxicity, including renal tubular necrosis, testicular degeneration, pancreatic injury, cardiac damage, and fibrosis in reproductive and accessory tissues, though the liver remained structurally intact. Overall, while both 100 mg/kg and 150 mg/kg dosages promoted reproductive and systemic benefits—with 150 mg/kg providing the most balanced and effective outcomes—the 200 mg/kg dose, despite achieving the highest semen indices, induced severe systemic toxicity. This identifies 150 mg/kg as the optimal and safest dose for enhancing male reproductive potential.

Sperm Parameters:

All experimental groups showed significant improvements in sperm count, motility, and morphology compared to the control group with 100mg, 150mg, & 200mg doses. (see Table 1). Significant at $p < 0.05$; significant at $p < 0.01$ compared to control.

Table-1: Effect of Zinc, Fructose, and Pumpkin Seed Supplementation (100 mg/kg Group, 150 mg/kg Group, 200 mg/kg Group) on Reproductive and Systemic Parameters in Albino Wistar Rats.

S.N.	Parameter	Control Group	100 mg/kg Group	150 mg/kg Group	200 mg/kg Group
1	**Sperm Count (million/mL)**	~70	75–76	100–118	120–122
2	**Motility (%)**	~60	65–68	76–84	88–92
3	**Viability (%)**	~80	82-85	86–92	94–96
4	**Morphology (%)**	~68	70–74	74–85	82–90
5	**Semen Volume (mL)**	~1.5	~1.7	~2.0	~2.0

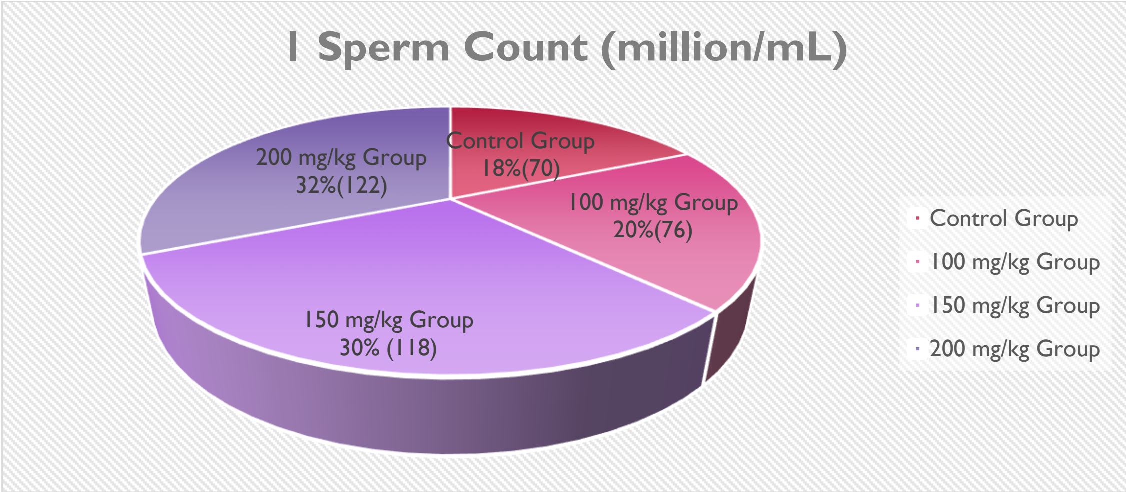


FIGURE-1: - To investigate Sperm Count by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.

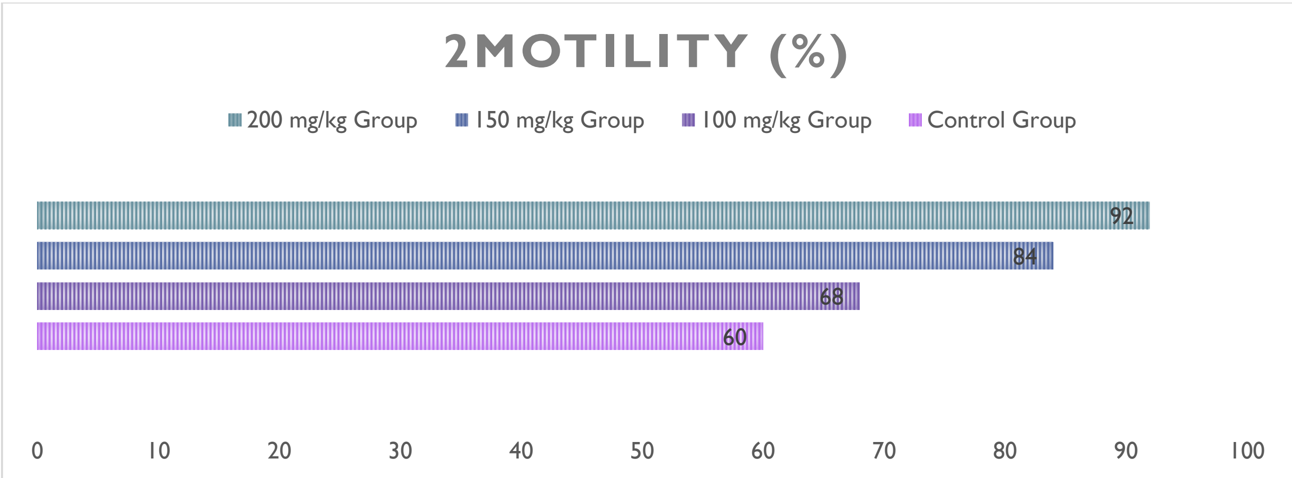


FIGURE-2: - To investigate Motility by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.

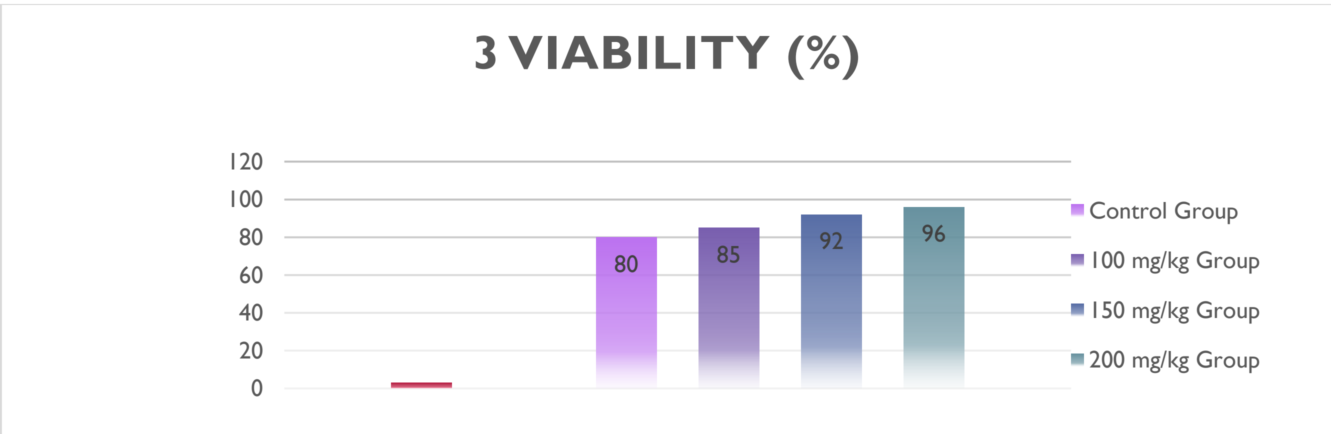


FIGURE-3: - To investigate the potential mechanisms Viability by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.

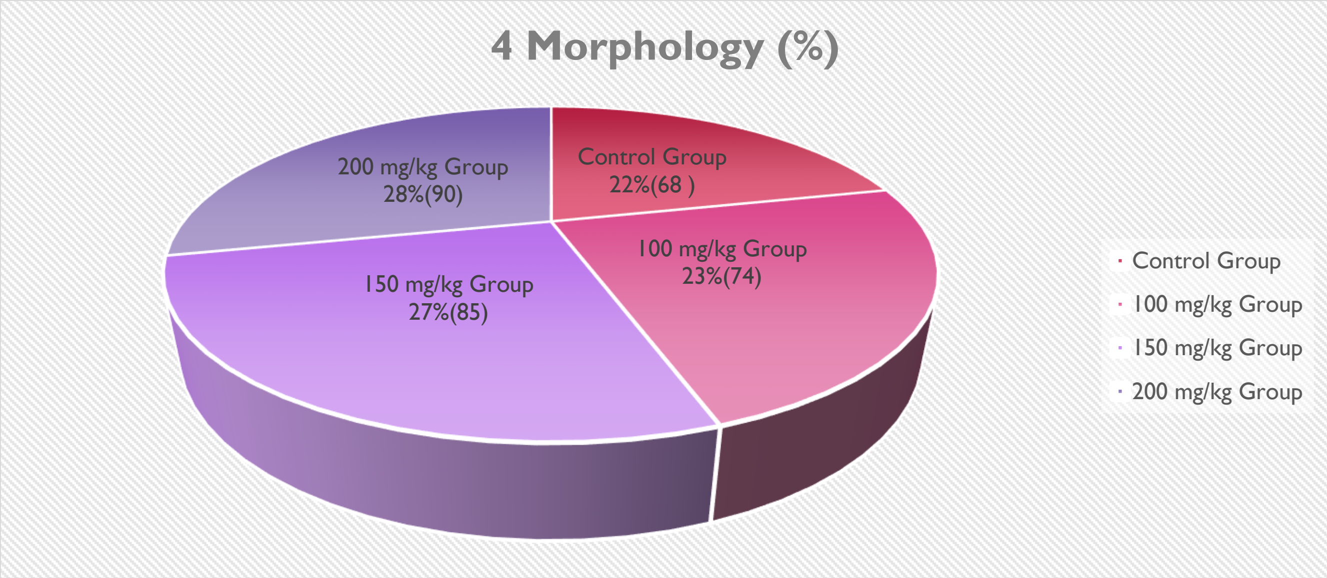


FIGURE4: - To investigate the Morphology by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.

Hormonal Analysis:

Serum testosterone levels increased significantly in all treated groups, with the highest increase observed in the 200 mg group (see Table 2 & Figure 4). This indicates that the combination positively influences hormonal balance essential for reproductive function.

Table 2: Testosterone levels increased significantly in all treated groups.

Group	Testosterone (ng/ml)
Control	Normal (~5.0)
100 mg	Normal (~5.0)
150 mg	7.2–7.6
200 mg	High (~9.0)

Significant at $p < 0.05$; significant at $p < 0.01$ compared to control.

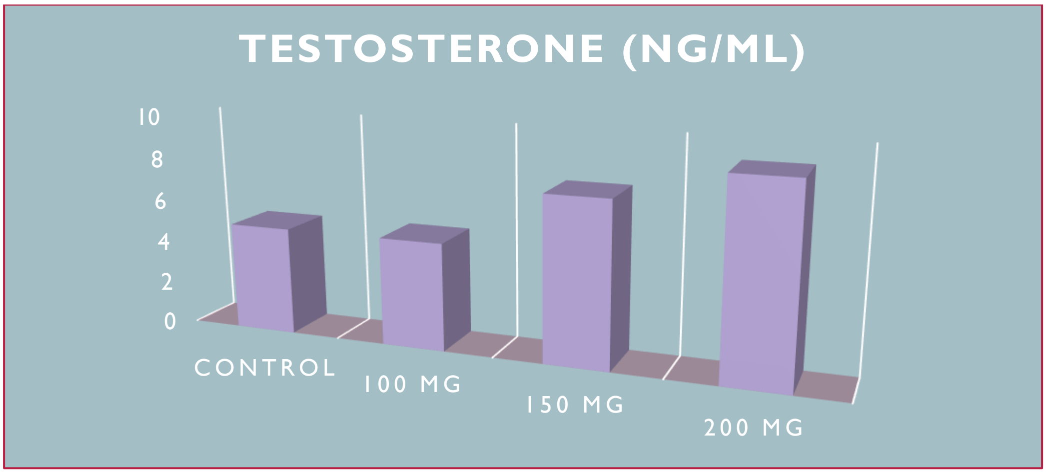
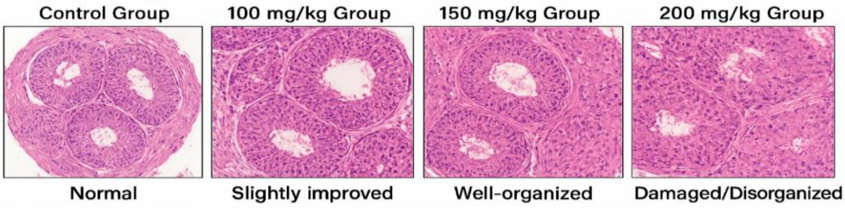


FIGURE-4: - To investigate the Testosterone levels by which a combination of zinc, fructose, and pumpkin seeds (with doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg) affects infertility-related diseases.

Histopathological Findings:

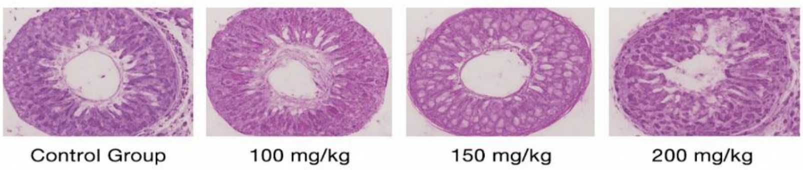
Histopathological examination of the testes revealed improved seminiferous tubule architecture, **testis, epididymis,** seminal vesicle and increased spermatogenesis in the treated groups,

Figure 5: **TESTIS HISTOLOGY**



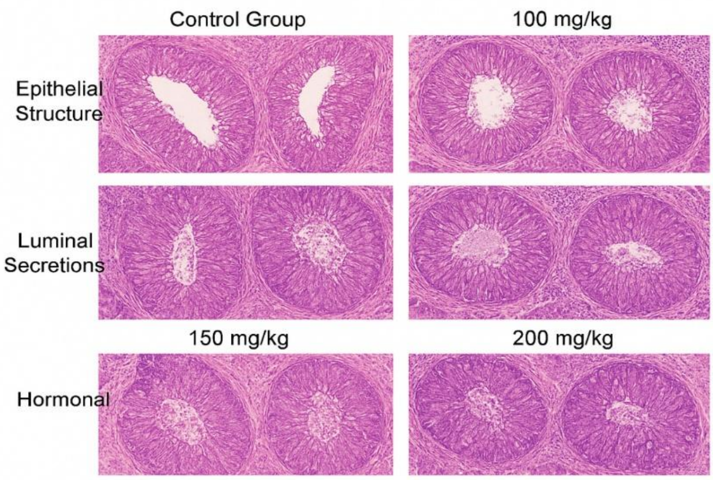
Seminiferous Tubule Structure	Normal	Decresed
Germ Cell Development	Improved	Decreased
Leydig Cell Activity	Signinantiuly improved	Poor develoment
Sertoli Cell Condition	Neimml	Decreased
Inflammation	Present	Damagedl
Degeneration of Germ Cells	Mnimmal	Fibrosis
Fibrosis	Slightly stresed	Damaged

Figure 6: EPIDIDYMIS HISTOLOGY



Parameter	100 mg/kg	150 mg/kg	200 mg/kg
Epithelial Lining	Normal	Mild mprovement	Disrupted
Luminal Sperm Density	Increased	Henressed	Reduced
Inflammation	Mild	Normalizel	Mlarncotion
Interstitial Spaces	Normalized	Normalized	Dilated damaged
Fibrosis	Fbrosis	Presence	Present
Overall Epididymal Integrity	Siightly altered	Significantly improve	Damaged

Figure7: SEMINAL VESICLE HISTOLOGY:



Eprithel Structure	Mild improvement	Improved	Significantly impoved
Luminal Secretions	Increaseced	Well-organed	Damaged Bou

4. DISCUSSION

The present study evaluated the reproductive and systemic effects of combined supplementation of zinc, fructose, and pumpkin seeds in male Wistar rats. The findings revealed a dose-dependent influence on sperm parameters, hormonal balance, and organ histopathology. The control group maintained baseline hematological, biochemical, and hormonal levels, though minor stress changes and slightly reduced sperm motility suggested natural physiological variation. Supplementation at 100 mg/kg produced noticeable improvements in semen quality, with higher sperm count, motility, and morphology compared to controls. Histological recovery across multiple organs and enhanced germ cell development indicated protective and restorative effects at this moderate dose. The 150 mg/kg group demonstrated the most favorable outcomes. This dosage improved reproductive parameters markedly, with sperm counts ranging from 100–118 million/mL, motility reaching 76–84%, and morphology between 74–85%. Hormonal analysis confirmed high-normal testosterone levels and balanced gonadotropins. Histopathology revealed preserved organ integrity, including hepatic, renal, cardiac, pulmonary, and reproductive tissues, along with evidence of vigorous spermatogenesis and enhanced Leydig cell activity. These results align with previous studies emphasizing the synergistic roles of zinc in spermatogenesis, fructose as an energy source for motility, and pumpkin seeds as antioxidants that counteract oxidative stress. In contrast, supplementation at 200 mg/kg, while yielding peak semen parameters (sperm count 120–122 million/mL, motility 88–92%, viability 94–96%), induced severe systemic toxicity. Histopathology showed renal tubular necrosis, testicular degeneration, cardiac damage, pancreatic lesions, and fibrosis in reproductive accessory organs. These adverse outcomes suggest that supraphysiologic stimulation leads to organ injury, overshadowing reproductive benefits. This aligns with literature indicating that excessive zinc intake disrupts redox homeostasis and that nutrient overload may trigger pathological changes rather than physiological benefits. Collectively, the results establish that while both 100 mg/kg and 150 mg/kg doses improve reproductive potential, the 150 mg/kg supplementation achieves an optimal balance between fertility enhancement and systemic safety. The findings underscore the importance of dose optimization in nutraceutical approaches to male fertility management.

5. CONCLUSION

This study demonstrates that supplementation with a combination of zinc, fructose, and pumpkin seeds enhances male reproductive health in a dose-dependent manner. The 100 mg/kg dose improved systemic and reproductive parameters moderately, while the 150 mg/kg dose produced the most optimal results, including significant improvements in sperm quality, hormonal balance, and organ histology without adverse effects. However, excessive supplementation at 200 mg/kg, despite yielding the highest semen indices, was associated with multi-organ toxicity, indicating the risks of supraphysiologic intake. Therefore, **150 mg/kg is identified as the most effective and safe therapeutic level** for improving male fertility in Wistar rats. These findings support the potential of combining micronutrients and natural compounds in fertility enhancement strategies. Future studies should explore molecular mechanisms, long-term outcomes, and translational potential in clinical settings.

6. ACKNOWLEDGMENT

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