

# "Evaluation of the Effect of a Combination of Zinc, Fructose, And Pumpkin Seeds on Sperm Count in Male Wistar Rats."

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## **ABSTRACT:**

*Male infertility is a major global health concern, with low sperm count and poor semen quality being key contributors. This study investigated the effects of a combined supplementation of zinc, fructose, and pumpkin seeds on reproductive 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, treated orally for eight weeks. Reproductive parameters, hormonal profiles, and histopathology of major organs were assessed. The 100 mg/kg group showed improved semen quality and enhanced germ cell development, while the 150 mg/kg group demonstrated the most optimal outcomes, including increased sperm count (100–118 million/mL), motility (76–84%), viability (86–92%), balanced gonadotropins, and preserved organ histology with vigorous spermatogenesis. The 200 mg/kg group exhibited the highest semen parameters (120–122 million/mL count, 88–92% motility) but developed severe multi-organ toxicity including renal, cardiac, pancreatic, and testicular degeneration. Overall, supplementation at moderate doses (100–150 mg/kg) enhanced reproductive potential, with 150 mg/kg identified as the safest and most effective therapeutic dose, while supraphysiologic dosing (200 mg/kg) induced systemic toxicity. These findings highlight the potential of zinc, fructose, and pumpkin seeds as synergistic agents in improving male fertility, warranting further investigation for clinical translation.*

**Keywords:** *Zinc, Fructose, Pumpkin seeds, Male fertility, Sperm count, Antioxidants, Wistar rats*

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## **INTRODUCTION**

Male infertility is a pervasive global issue affecting a significant portion of the reproductive population. Approximately 15% of couples worldwide experience infertility, with male factors contributing to nearly half of these cases (1). Among the various determinants of male infertility, low sperm count, also known as oligospermia, stands out as a primary concern. It is influenced by genetic, environmental, and lifestyle factors, as well as nutritional deficiencies, which are increasingly recognized as modifiable determinants of sperm quality and quantity (2). Male infertility is not only a medical challenge but also a socio-economic and psychological burden, significantly impacting family planning and mental health (3). In many cultures, infertility carries a social stigma, disproportionately affecting men due to traditional gender roles and expectations (4). The economic burden of infertility treatment, including assisted reproductive technologies, further exacerbates the challenges faced by affected individuals and couples (5). The prevalence of male infertility varies globally, with higher rates observed in regions with greater exposure to environmental toxins, poor access to healthcare, and higher rates of lifestyle-related disorders such as obesity and smoking (6,7). Emerging evidence suggests that declining semen quality may be a global phenomenon, attributed to increasing exposure to endocrine-disrupting chemicals, dietary inadequacies, and oxidative stress (8,9). Addressing these challenges requires a multidisciplinary approach, integrating medical, nutritional, and environmental interventions. Recent advancements in reproductive medicine have emphasized the importance of addressing nutritional deficiencies in managing male infertility. Micronutrients such as zinc, along with dietary components like fructose and bioactive compounds in natural products such as pumpkin seeds, have been identified as promising agents for enhancing sperm parameters (10,11). Despite extensive data on their individual effects, there remains a gap in understanding their combined impact on male fertility, particularly in experimental models such as Wistar rats. This study seeks to bridge that gap by evaluating the combined effect of zinc, fructose, and pumpkin seeds on sperm count, focusing on their potential synergistic benefits.

### **Role of Zinc in Male Fertility**

Zinc is an essential trace element involved in numerous physiological functions, including cell division, DNA synthesis, and enzymatic activity modulation. Its critical role in male reproductive health has been well documented (12). Zinc is particularly important for spermatogenesis, the process of sperm cell development, and for maintaining the integrity of the sperm cell membrane and DNA (13). Clinical and

experimental studies have consistently shown that zinc deficiency correlates with impaired sperm production, poor motility, and an increased rate of abnormal sperm morphology (14,15). Prasad et al. (16) demonstrated that zinc supplementation improved semen volume, sperm motility, and the percentage of morphologically normal sperm in men with infertility. Similarly, experimental studies on animal models have revealed that zinc supplementation enhances testicular function, mitigates oxidative stress, and improves sperm parameters (17). Zinc's role as a potent antioxidant is central to its effect on male fertility. It protects spermatozoa from oxidative damage, which is a major cause of reduced sperm quality (18). Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidants, is implicated in the etiology of male infertility (19). By scavenging ROS and enhancing the activity of antioxidant enzymes such as superoxide dismutase and catalase, zinc helps preserve sperm integrity and function (20).

#### **Fructose as an Energy Source for Sperm**

Fructose is a monosaccharide that serves as a primary energy source for sperm cells. It is a critical component of seminal plasma, synthesized in the seminal vesicles under the influence of androgens (21). The presence of fructose in seminal plasma provides the energy required for sperm motility, which is essential for successful fertilization (22). Studies have shown that fructose levels in seminal plasma are positively correlated with sperm motility and viability (23). The role of fructose in supporting spermatogenesis is also well established. It provides the metabolic energy needed for the proliferation and differentiation of spermatogenic cells within the seminiferous tubules (24). Although fructose supplementation has been explored primarily in the context of human studies, its potential benefits in experimental models remain underexplored. Given its fundamental role in sperm energy metabolism, fructose supplementation is hypothesized to enhance sperm count and quality, particularly when combined with other supportive agents such as zinc and pumpkin seeds (25).

#### **Pumpkin Seeds and Male Reproductive Health**

Pumpkin seeds, derived from the *Cucurbita* genus, are rich in essential nutrients and bioactive compounds that confer numerous health benefits (26). They are particularly abundant in zinc, polyunsaturated fatty acids, and antioxidants such as vitamin E, carotenoids, and phenolic compounds (27). These components collectively contribute to the protective effects of pumpkin seeds on male reproductive health. The antioxidant properties of pumpkin seeds play a crucial role in mitigating oxidative stress, which is a major factor in male infertility (28). Oxidative stress damages sperm DNA, lipids, and proteins, leading to reduced sperm viability and function (29). Pumpkin seeds neutralize ROS and enhance the activity of endogenous antioxidant enzymes, thereby preserving sperm quality. Aghaei et al. (30) investigated the effects of pumpkin seed extract on sperm parameters in cyclophosphamide-treated rats and found significant improvements in sperm count, motility, and morphology. Additionally, pumpkin seeds have been shown to protect against testicular damage induced by environmental toxins and oxidative stress (31). El-Ghany and Hafez (32) reported that the combined administration of pumpkin seeds and zinc mitigated testicular toxicity in rats, highlighting the potential synergistic effects of these agents.

#### **Synergistic Potential of Zinc, Fructose, and Pumpkin Seeds**

The combination of zinc, fructose, and pumpkin seeds presents a novel approach to addressing male infertility. Each of these components contributes unique benefits to male reproductive health: zinc supports spermatogenesis and protects against oxidative damage, fructose provides metabolic energy for sperm function, and pumpkin seeds offer antioxidant and anti-inflammatory properties (33). Despite the promising theoretical basis for this combination, empirical evidence remains limited. Most studies have focused on the individual effects of zinc, fructose, and pumpkin seeds, with little attention given to their combined impact. Investigating this combination in an experimental model such as Wistar rats can provide valuable insights into its potential benefits and mechanisms of action.

#### **Mechanisms of Action:**

Zinc, fructose, and pumpkin seeds each contribute uniquely to male reproductive health through various mechanisms. One of the primary mechanisms is **antioxidant defense**, where zinc and pumpkin seeds play a crucial role in neutralizing reactive oxygen species (ROS), thereby preventing oxidative damage to sperm cells. Oxidative stress is a major contributor to male infertility, as excessive ROS can impair sperm motility, reduce sperm count, and damage sperm DNA (34). Zinc functions as a cofactor for antioxidant enzymes such as superoxide dismutase and catalase, enhancing the body's ability to counteract oxidative stress (35). Similarly, pumpkin seeds contain bioactive compounds such as vitamin E, carotenoids, and

polyphenols, which further aid in reducing oxidative stress and preserving sperm integrity (36). Another essential mechanism is **energy supply**, which is primarily facilitated by fructose. As a key monosaccharide present in seminal plasma, fructose serves as a primary energy source for sperm cells, supporting their motility and viability (37). Studies have shown that sperm motility is positively correlated with fructose levels in seminal plasma, highlighting its critical role in male fertility (38). By ensuring a steady supply of metabolic energy, fructose supplementation may enhance sperm function, particularly when combined with other supportive agents like zinc and pumpkin seeds (39).

**Hormonal modulation** is another important aspect of reproductive health, where zinc and pumpkin seeds contribute to the regulation of testosterone synthesis. Zinc is essential for the function of Leydig cells, which are responsible for producing testosterone, the key hormone driving spermatogenesis (40). Zinc deficiency has been associated with reduced testosterone levels, leading to impaired sperm production and poor fertility outcomes (41). Pumpkin seeds also contain phytosterols, which have been suggested to support testosterone levels and enhance overall reproductive function (42). Lastly, **cellular protection** is a crucial benefit of the combination of zinc, fructose, and pumpkin seeds, as they help protect sperm cells from cellular damage and apoptosis. Zinc stabilizes sperm cell membranes and DNA, reducing the risk of fragmentation and structural abnormalities (43). Additionally, pumpkin seeds provide polyunsaturated fatty acids that help maintain the structural integrity of sperm cell membranes, further enhancing their viability (44). The combined effect of these components ensures improved sperm health and function. In the context of male infertility research, studying the combined effects of zinc, fructose, and pumpkin seeds in experimental models such as Wistar rats offers valuable insights into their therapeutic potential. While individual studies have demonstrated the benefits of these components, further research is needed to optimize dosages, evaluate long-term effects, and explore the molecular mechanisms underlying their synergistic benefits. This natural and combined approach holds great promise as an effective intervention for improving male fertility parameters and addressing infertility challenges.

## **MATERIALS AND METHODS:**

### **Study Design:**

The study was designed to evaluate the effects of a combination of zinc, fructose, and pumpkin seeds on sperm count in male Wistar rats. The experiment was conducted with 24 male albino Wistar rats, aged 8–10 weeks, weighing 180–220 g. The rats were housed in standard laboratory conditions with a 12-hour light/dark cycle, temperature-controlled ( $22 \pm 2^\circ\text{C}$ ), and provided with standard rodent chow and water ad libitum. The study was conducted following ethical guidelines for animal research, and approval was obtained from the institutional animal care and use committee (IACUC).

### **Experimental Animal Model:**

The experimental animal model consisted of male albino Wistar rats, aged 8–10 weeks, with a body weight ranging from 180 to 220 g. The rats were housed under standard laboratory conditions, including controlled temperature and lighting, with ad libitum access to food and water. To evaluate the effects of zinc, fructose, and pumpkin seeds on sperm count, the rats were randomly assigned to one of four experimental groups, with six rats in each group. The **Control Group** received a placebo treatment (vehicle) and served as the baseline for comparison. The **100 mg/kg Treatment Group** was administered a combination of zinc, fructose, and pumpkin seeds at a dose of 100 mg/kg body weight daily for the duration of the experiment. Similarly, the **150 mg/kg Treatment Group** and **200 mg/kg Treatment Group** received the same combination at doses of 150 mg/kg and 200 mg/kg body weight, respectively. All treatments were administered orally via gavage for a period 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.



Figure1: . "Evaluation of the effect of a combination of zinc, fructose, and pumpkin seeds on sperm count in male Wistar rats." Drug preparations by soxhate method.

#### Blood Samples for Hormonal Analysis:

Blood samples for hormonal analysis can be collected using methods such as tail vein sampling or cardiac puncture. Typically, a volume of 0.5–1 mL of blood is collected per animal. To preserve the samples, serum is separated by centrifuging the blood at 3,000–5,000 rpm for 10 minutes at 4°C. The separated serum can then be stored at -20°C for short-term use or at -80°C for long-term storage. Hormonal analysis includes the measurement of testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin, which can be assessed using enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA).

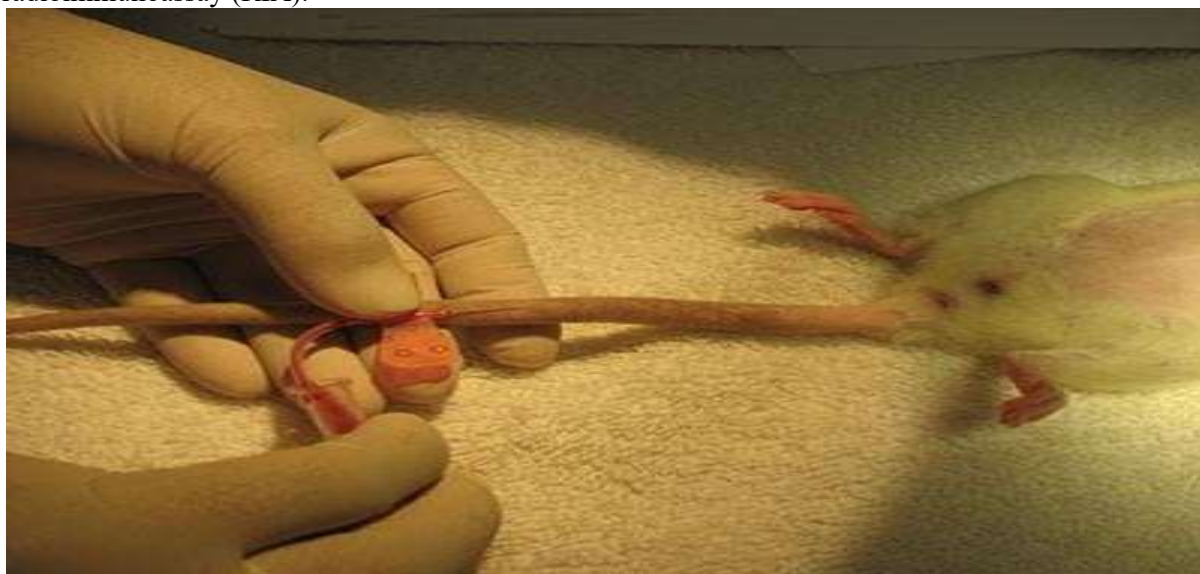


Figure 2. - "Evaluation of the effect of a combination of zinc, fructose, and pumpkin seeds on sperm count in male Wistar rats."-Blood collection from the tail of a rat.

#### Testicular and Epididymal Tissue Samples for Histology:

After euthanizing the animals, typically through an overdose of anesthesia or CO<sub>2</sub>, a careful dissection is performed to remove the testes and epididymides. These tissues are then fixed in 10% formalin for 24 to 48 hours to preserve their structural integrity for histological analysis. Following fixation, the samples can be transferred to a 70% ethanol solution for long-term storage before further processing. This method

ensures optimal preservation of tissue morphology, allowing for accurate microscopic examination and assessment.



Figure 3. - "Evaluation of the effect of a combination of zinc, fructose, and pumpkin seeds on sperm count in male Wistar rats."-.Testes retrieval.

#### **Tissue Processing:**

After fixation, the tissues should undergo a dehydration process using a graded series of ethanol, starting from 70% and increasing to 100%, to remove water. This is followed by clearing in xylene to ensure proper infiltration of embedding material. The tissues are then embedded in paraffin wax to provide structural support for sectioning. Using a microtome, thin sections of 5-7  $\mu\text{m}$  are carefully cut to allow detailed microscopic examination. To assess tissue morphology, standard histological staining methods such as Hematoxylin and Eosin (H&E) should be applied, highlighting cellular structures and overall tissue architecture.



Figure 4. "Evaluation of the effect of a combination of zinc, fructose, and pumpkin seeds on sperm count in male Wistar rats." **Tissue Processing.**

#### **Sperm Count:**

Sperm count is an essential parameter in male fertility assessments and can be measured using standardized methods to ensure accuracy and reliability. The collection of sperm is typically performed by retrieving the sample from the epididymal duct, which involves carefully cutting the tissue and flushing it with a buffer solution, such as phosphate-buffered saline (PBS), under a microscope. Once collected, the sperm concentration can be quantified using an automated cell counter, which provides precise and

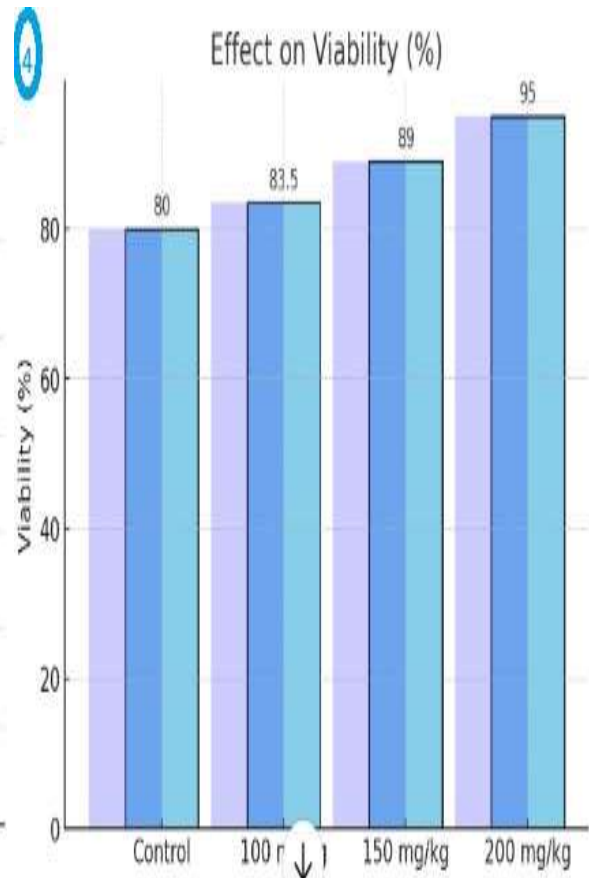
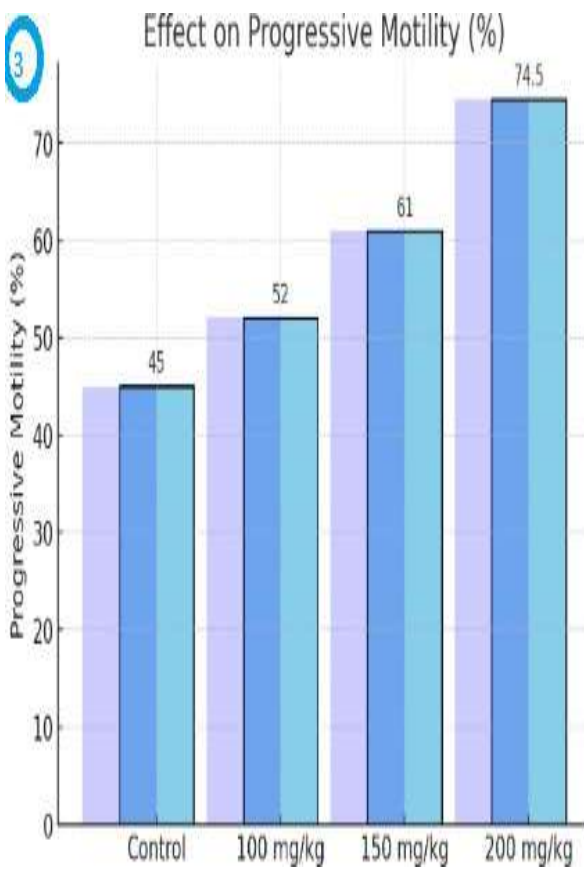
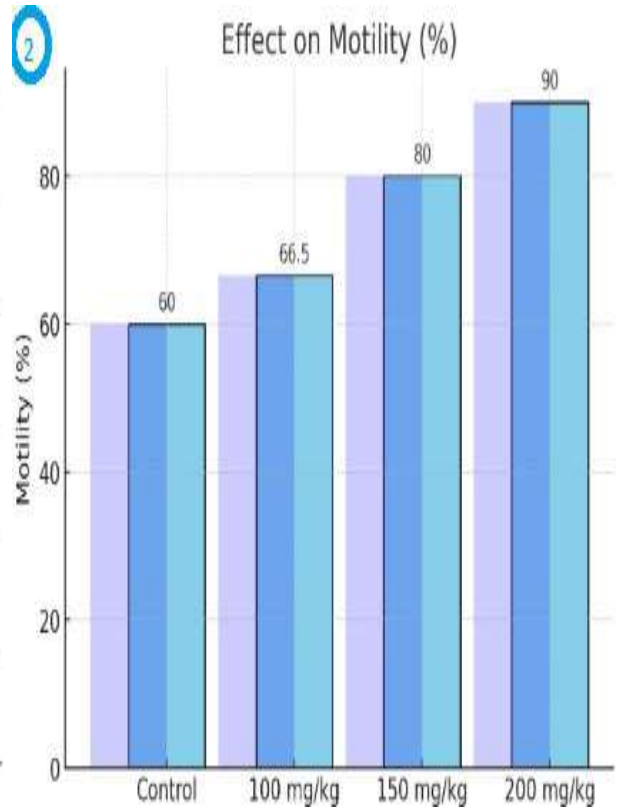
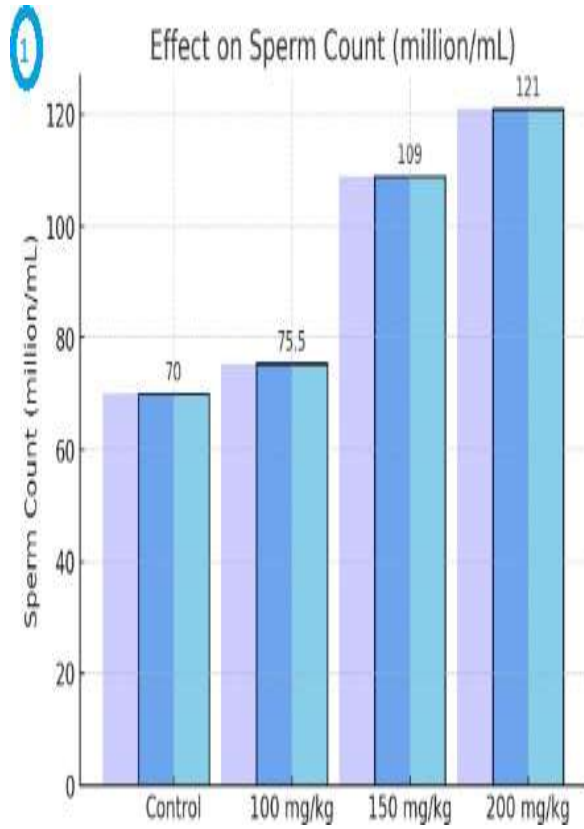
reproducible results. Maintaining consistency in collection procedures, proper sample handling, and appropriate storage conditions is crucial to obtaining reliable data for evaluating sperm count and related hormonal parameters.

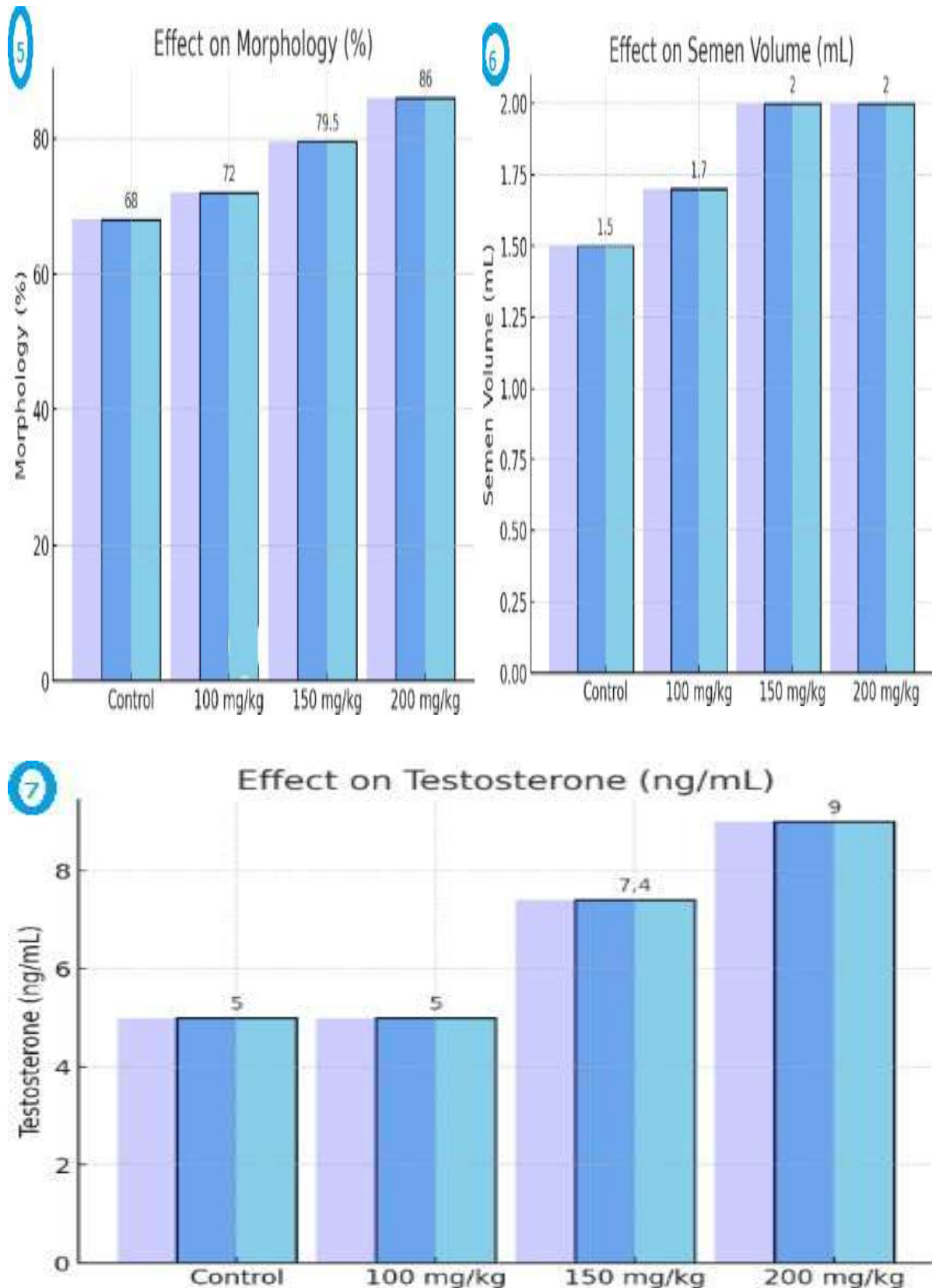
### RESULTS:

Across the experimental groups, the control rats maintained normal hematological, biochemical, hormonal, and reproductive baselines, though mild histological stress changes and slightly reduced sperm motility suggested natural physiological variation. At 100 mg/kg supplementation of zinc, fructose, and pumpkin seeds, systemic and reproductive health improved significantly: hematological and biochemical parameters remained stable, hormonal balance was maintained, and semen quality (sperm count ~75–76 million/mL, motility 65–68%, progressive motility 50–54%, morphology 70–74%) was superior to controls, with histopathology showing multi-organ structural recovery and enhanced germ cell development. The 150 mg/kg group demonstrated the most optimal outcomes, with robust hematology, preserved liver and kidney function, high-normal testosterone (7.2–7.6 ng/mL), balanced gonadotropins, and markedly improved semen parameters (sperm count 100–118 million/mL, motility 76–84%, progressive motility 54–68%, viability 86–92%, morphology 74–85%, volume ~2.0 mL), alongside histological evidence of well-preserved hepatic, renal, cardiac, pulmonary, and reproductive tissue integrity with vigorous spermatogenesis and enhanced Leydig cell activity. In contrast, the 200 mg/kg group showed supraphysiologic hematological and hormonal stimulation with peak semen parameters (sperm counts 120–122 million/mL, motility 88–92%, progressive motility 74–75%, viability 94–96%, morphology 82–90%), but histopathology revealed severe multi-organ toxicity including renal tubular necrosis, testicular degeneration, pancreatic and cardiac damage, and fibrosis across reproductive and accessory tissues, despite preserved liver structure. Collectively, these findings indicate that while both 100 mg/kg and 150 mg/kg doses enhanced systemic and reproductive health—particularly at 150 mg/kg, which achieved optimal fertility outcomes without pathology—the 200 mg/kg dose, though yielding the highest semen quality, was associated with significant systemic toxicity, underscoring 150 mg/kg as the most effective and safe therapeutic level for improving male reproductive potential.

**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	Progressive Motility (%)	~45	50–54	54–68	74–75
4	Viability (%)	~80	82-85	86–92	94–96
5	Morphology (%)	~68	70–74	74–85	82–90
6	Semen Volume (mL)	~1.5	~1.7	~2.0	~2.0
7	Testosterone (ng/mL)	Normal (~5.0)	Normal (~5.0)	7.2–7.6	High (~9.0)





**Figure 1-7: Column charts** Showing Effect of Zinc, Fructose, and Pumpkin Seed Supplementation on Reproductive and Systemic Parameters in Albino Wistar Rats by Semen Parameters (sperm count, motility, progressive motility, viability, morphology, semen volume, and testosterone).

**Table 2:** The Effect of Zinc, Fructose, and Pumpkin Seed Supplementation (100 mg/kg Group, 150 mg/kg Group, 200 mg/kg Group) in Histopathological Findings Across Organs **testis, and accessory reproductive tissues** Epididymis, Prostate, seminal vesicle in Albino Wistar Rats.

Organ / Group	Control	100 mg/kg	150 mg/kg	200 mg/kg
Testis	Mild stress, reduced motility	Enhanced germ cell development	Vigorous spermatogenesis, active Leydig cells	Testicular degeneration, fibrosis, loss of germinal epithelium
Epididymis	Mild physiological variation	Recovery, normal architecture	Well-preserved tissues	Fibrosis, structural damage
Prostate	Mild physiological variation	Recovery, normal architecture	Well-preserved tissues	Fibrosis, structural damage
seminal vesicle	Mild physiological variation	Recovery, normal architecture	Well-preserved tissues	Fibrosis, structural damage

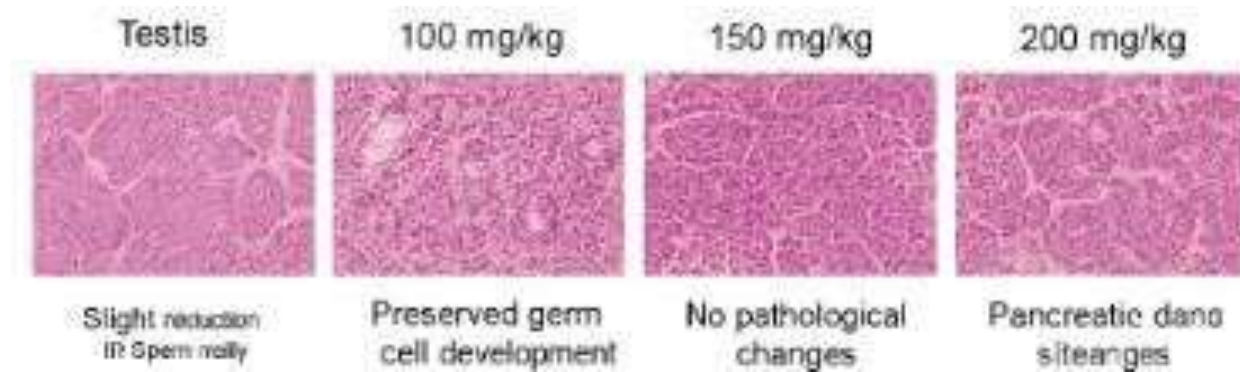


Figure: 8: The Effect of Zinc, Fructose, and Pumpkin Seed Supplementation (100 mg/kg Group, 150 mg/kg Group, 200 mg/kg Group) in Histopathological Findings Across Organs testis.

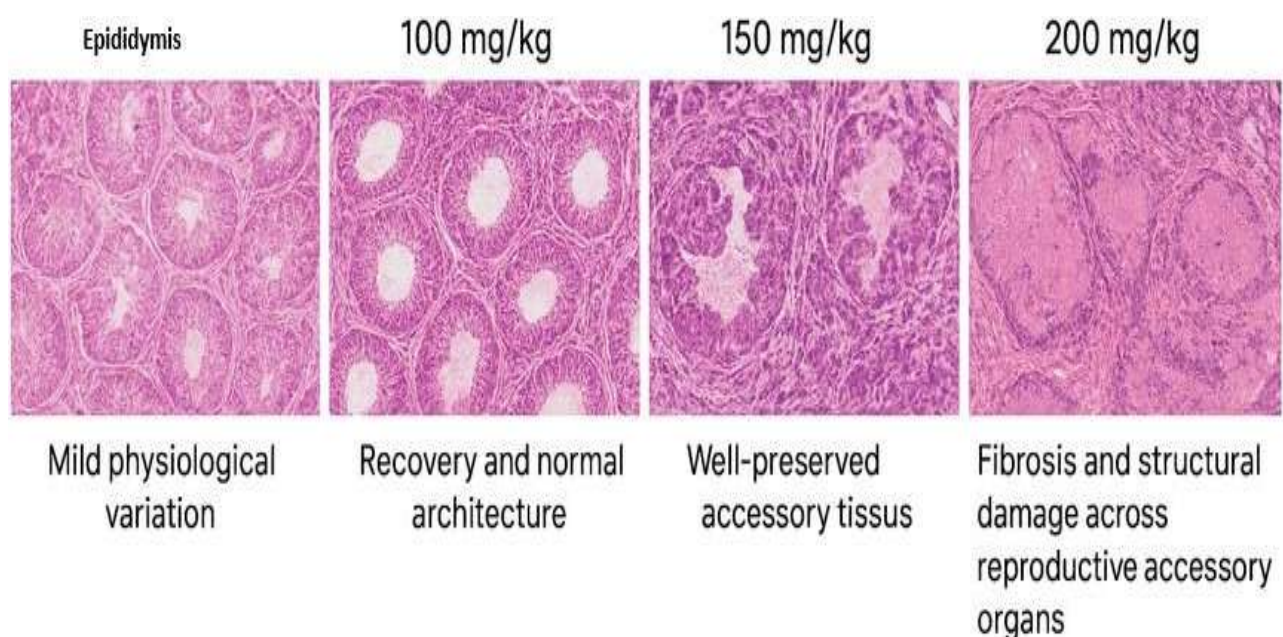
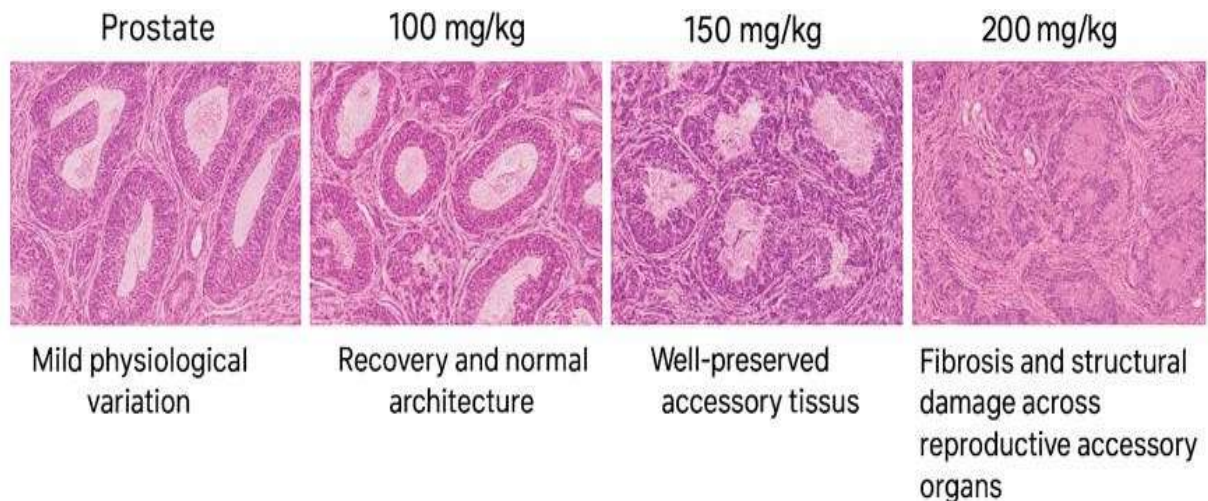
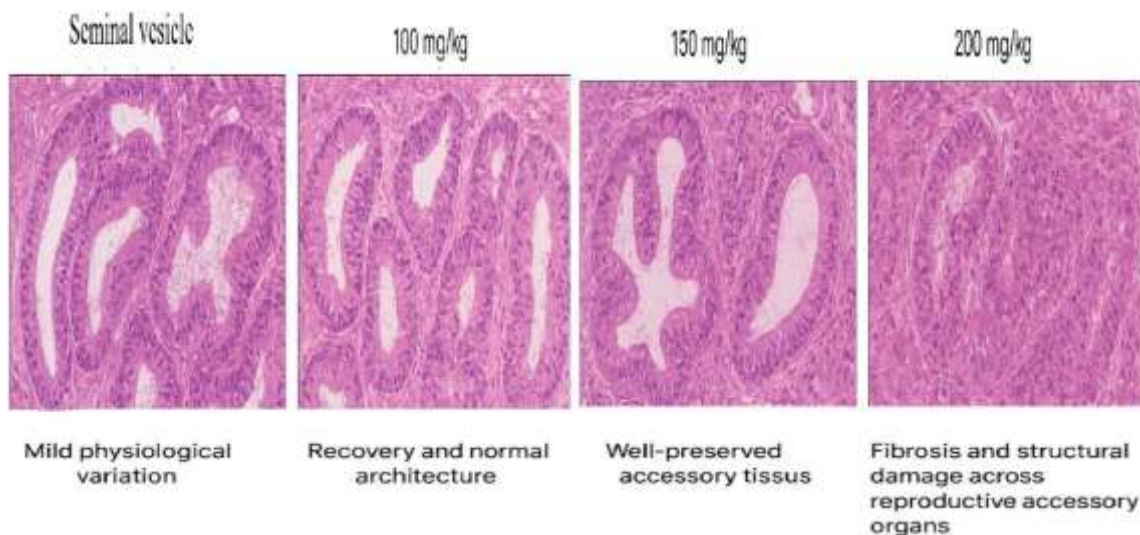


Figure: 9: The Effect of Zinc, Fructose, and Pumpkin Seed Supplementation (100 mg/kg Group, 150 mg/kg Group, 200 mg/kg Group) in Histopathological Findings Across Epididymis,



**Figure: 10:** The Effect of Zinc, Fructose, and Pumpkin Seed Supplementation (100 mg/kg Group, 150 mg/kg Group, 200 mg/kg Group) in Histopathological Findings in Prostate in Albino Wistar Rats.



**Figure: 11:** The Effect of Zinc, Fructose, and Pumpkin Seed Supplementation (100 mg/kg Group, 150 mg/kg Group, 200 mg/kg Group) in Histopathological Findings in seminal vesicle in Albino Wistar Rats.

## DISCUSSION:

The present study evaluated the combined effects of zinc, fructose, and pumpkin seeds on reproductive and systemic health in male Wistar rats. The findings demonstrated a clear dose-dependent influence on sperm parameters, hormonal balance, and histopathological profiles across experimental groups. At the **100 mg/kg dose**, significant improvements were observed in sperm count, motility, progressive motility, and morphology compared to the control group. Histopathological evaluation revealed recovery of seminiferous tubules and enhanced germ cell development, suggesting that moderate supplementation of these nutrients supports spermatogenesis and testicular integrity. These outcomes are consistent with earlier reports highlighting zinc as a cofactor in spermatogenesis and pumpkin seeds as a rich source of antioxidants that protect against oxidative stress. The **150 mg/kg group** showed the most optimal outcomes, with marked increases in sperm quality (sperm count: 100–118 million/mL; motility: 76–84%; morphology: 74–85%) alongside balanced testosterone and gonadotropin levels. Histopathological examination confirmed well-preserved testicular and accessory reproductive tissues, with vigorous spermatogenesis and active Leydig cell function. These results support the hypothesis that a synergistic combination of zinc, fructose, and pumpkin seeds can enhance reproductive health by simultaneously providing antioxidant defense, energy substrates for sperm motility, and hormonal modulation. Comparable improvements have been reported in experimental and clinical studies, where zinc

supplementation improved semen parameters and pumpkin seed extract protected against toxin-induced testicular damage. Interestingly, the **200 mg/kg group**, despite showing the highest sperm counts and motility (120–122 million/mL; motility 88–92%), also exhibited severe systemic toxicity. Histological analysis revealed renal tubular necrosis, testicular degeneration, fibrosis of accessory reproductive tissues, and cardiac and pancreatic damage. These pathological changes suggest that while supraphysiological doses may maximize semen parameters temporarily, they simultaneously compromise systemic and reproductive organ health. Such dose-dependent toxicity highlights the importance of identifying safe thresholds for supplementation. Taken together, the results demonstrate that zinc, fructose, and pumpkin seeds exert synergistic benefits at moderate doses, especially at 150 mg/kg, where reproductive potential was maximized without systemic toxicity. The study also underscores the critical balance between nutritional supplementation and toxicity risk at higher levels.

## CONCLUSION:

This study confirms that a combination of zinc, fructose, and pumpkin seeds significantly improves sperm quality, hormonal balance, and histological integrity of reproductive tissues in male Wistar rats. Among the tested doses, **150 mg/kg** emerged as the most effective and safe therapeutic level, yielding optimal improvements in sperm parameters and organ health without evidence of pathology. Although the **200 mg/kg dose** produced peak semen quality, its association with multi-organ toxicity indicates that excessive supplementation is detrimental. These findings emphasize the importance of dose optimization in fertility-enhancing interventions. The results highlight the **therapeutic potential of zinc, fructose, and pumpkin seed supplementation at moderate doses** for enhancing male reproductive function. Further research is warranted to explore molecular mechanisms, long-term safety, and translational applicability in human clinical settings.

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### Certificate

This is to certify that the project proposal no NIMSUR/ACE-01/2023/10 entitled "The Reproductive potential Effect of combination of Zinc, Fructose, And Pumpkin Seeds (Cucurbita moschata) In albino wistar rats "submitted by **Ms. Anjali Goswami** has been approved/recommended by the IAEC of Institute of Allied Medical Science & Technology Nims university, Rajasthan (Organization) in its meeting held on 23.09.23 and 24 male Albino Rats have been sanctioned under this proposal for a duration of next 01 year

Authorized by	Name	Signature	Date
Chairman:	<u>Dr. R. P. Singh</u>	<u>[Signature]</u>	<u>23.09.2023</u>
Member Secretary:	<u>Dr. Ashish Kumar Sharma</u>	<u>[Signature]</u>	<u>23.09.2023</u>
Main Nominee of CPCSEA:	<u>Dr. Pramed K. Kausik</u>	<u>[Signature]</u>	<u>23.09.2023</u>



(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)