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Dose- Dependent Effects of Biosynthesized Selenium Nanoparticles on Histopathology in Rats

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Abstract

The study aimed to estimate the effect of biosynthesized selenium nanoparticles (SeNPs) on some parameters of male reproductive efficacy and histological changes in the testis of immature male rats to accelerate the maturation of the male reproductive system. The experiment utilized 40 immature male rats aged 1 month, divided into five groups as follows: the control group received D.W, G1, G2, G3, and G4 received 50, 100, 200, and 400 µg/Kg B.W of SeNps daily for 30 days. After that, the animals of the experiment were sacrificed to obtain the testis, one used to estimate the weight of the testis and to calculate the count and live/dead ratio of sperm. The other testis was kept in formalin to be sectioned for histological study. The results of the study revealed a significant increase in sperm count, live/dead ratio, and testicular weight in some treated groups as compared with the control. The histological study showed a well-organized germinal layer with an increase in interstitial tissue and an increase in number of spermatozoa with a significant increase in the maturation of seminiferous tubules, the diameter of seminiferous tubules, and germinal layer thickness in all treated groups.

Conclusion: the green synthesis SeNPs caused an increase in the maturation of the immature male reproductive system in rats.

Keywords: Selenium, nanoparticles, Male fertility

الملخص

هدفت هذه الدراسة إلى تقدير تأثير جسيمات السيلينيوم النانوية المُصنّعة حيويًا على بعض مؤشرات كفاءة الجهاز التناسلي الذكري والتغيرات النسيجية في خصيتي ذكور الجرذان غير البالغة، وذلك بهدف تسريع نضج الجهاز التناسلي الذكري. استُخدم في التجربة 40 فأرًا ذكرًا غير بالغ، يبلغ عمره شهرًا واحدًا، قُسّمت إلى خمس مجموعات كما يلي: تلقت المجموعة الضابطة الماء المقطر، وتلقت المجموعات الأخرى **G_1,G_2,G_3,G_4** جرعات يومية من جسيمات السيلينيوم النانوي 50و100و200و400 ميكروغرام/كيلوغرام من وزن الجسم على التوالي، لمدة 30 يومًا. بعد ذلك، تم تضحية حيوانات التجربة للحصول على الخصيتين، حيث استُخدمت إحداهما لتقدير وزن الخصية وحساب عدد الحيوانات المنوية ونسبة الحيوانات المنوية الحية إلى الميتة. أما الخصية الأخرى، فقد حُفظت في الفورمالين لتقطيعها ودراستها نسيجيًا. أظهرت نتائج الدراسة زيادة ملحوظة في عدد الحيوانات المنوية، ونسبة الحيوانات المنوية الحية إلى الميتة، ووزن الخصية في بعض المجموعات المُعالجة مقارنةً بالمجموعة الضابطة. أظهرت الدراسة النسيجية طبقة جرثومية منظمة جيدًا مع زيادة في النسيج الخلالي وزيادة في عدد الحيوانات المنوية، بالإضافة إلى زيادة ملحوظة في نضج الأنابيب المنوية، وقطرها، وسماكة الطبقة الجرثومية في جميع المجموعات المعالجة.

الكلمات المفتاحية :السيلينيوم، الجسيمات النانوية، خصوبة الرجال

Introduction

The male reproductive system consists of testes, reproductive ducts, and sex glands[1] maturation and physiological regulation of the male reproductive system are under control of the hypothalamic, pituitary, and gonadal (HPG) axis[2], in which the hypothalamus produces hormone which regulates pituitary gonadotropins (LH and FSH) production, thereby modulating Leydig, and Sertoli cell function, androgen production, and male reproductive system fertility [3]; [4],[5].

Selenium (Se) is an essential trace element that is present as selenocysteine in a family of selenoproteins, many of which play an important role in redox regulation and endocrine homeostasis. Selenoprotein families include glutathione peroxidases (GPXs), glutathione reductases (GRs), and Selenoprotein P. These proteins collectively support antioxidant defense and thyroid hormone metabolism, the two processes that are essential for male reproductive function [6], [7].

Nanotechnology refers to particles with a size of 1-100 nanometers . Selenium nanoparticles (SeNPs) have emerged as a promising class of nanomaterials due to their excellent biocompatibility and comparatively

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low toxicity (compared with certain other nanomaterials). Recent reviews highlight their potential in antioxidant. anti-inflammatory [8]. antimicrobial [9], and anticancer applications [10], with growing interest in biomedical and translational uses [11].

Spermatogenesis is a term that refers to the cellular divisions and developmental changes that happen in the seminiferous tubules of the testes, by which male germ cells develop into mature spermatozoa within the seminiferous tubules of the testes [12],[13]. The process is supported by somatic cells, particularly Sertoli, Leydig, and peritubular myoid cells. [4], [12]. During spermiogenesis, major changes include chromatin condensation, acrosome formation, and flagellum development [14], [12]. Mature spermatozoa consist of a head (containing the nucleus and acrosome) and a flagellum (tail), which is essential for motility [14].

. In rats, no period of testicular quiescence can stop the reproductive gland secretion. The maturation of the male reproductive system and development takes place at an early age and in four stages. [15], [16]. The stages are the neonatal period began from birth to 7 days of age, the infantile period, from 8 to 21 days, the juvenile period, extending to 35 days, and the peripubertal period, extending to 55 – 60 days. These stages end when mature spermatozoa are present in the vas deferens.

Material and Methods

Forty immature male white laboratory rats (*Rattus norvegicus*) weighing 100 ± 5 grams at one month of age were kept at the room temperature 25±2 Cº, 12 hours of light per day from four lid lamps, a humidity level of between 40-50%, and daily access to food and water.

The Nano-selenium particles were synthesized by use of starch as a stabilizing agent and ascorbic acid as a reducing agent according to [17]. The characterization of the synthesized selenium nanoparticles was diagnosed by visual observation (color change from aqueous to pale yellow, then to dark brown[18]. The second test is the Fourier transform infrared spectroscopy (FTIR) [19]. The third is the zeta potential [20], and the fourth is X-Ray diffraction [21].

The animals were divided into five groups with 8 male rats in each group. The five groups were given a daily oral single dose for 30 treatment days as follows: Group 1 (control): Administered orally 0.2 ml of D.w. Group 2: Administered orally 50 µg/kg BW of SeNPs daily. Group 3: Administered orally 100 µg/kg of SeNPs daily. Group 4: Administered orally 200 µg/kg BW of SeNPs daily. Group 5: Administered orally 400 µg/kg BW of SeNPs daily. The experimental rats were sacrificed after 30 days of experiment to obtain the testis to study the testicular weight, dead/ live ratio and count of sperms. Also, one testis was kept in 10% formalin for histological dissecting to evaluate the histological changes and histometry, including (maturation of seminiferous tubules, thickness of interstitial layer, and diameter of seminiferous tubules)

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Results

Male Reproductive Parameters Assays:

Table 1 Effect of Nano Selenium dosage(µg/kg) on Male reproductive traits in rats (Mean±SE) N=8

Dosages	Sperm count x10⁶	Live/dead ratio %	Testicle weight/g
Control	1.83 ± 0.217 C	92.750 ± 1.359 B	1.316 ± 0.021 C
G1	2.72 ± 0.086 C	97.750 ± 1.221 A	1.673 ± 0.048 B
G2	3.62 ± 0.151 C	98.750 ± 0.839 A	1.833 ± 0.035 A
G3	6.19 ± 0.933 B	97.500 ± 1.636 A	1.812 ± 0.037 A
G4	13.38 ± 1.665 A	99.750 ± 0.250 A	1.627 ± 0.042 B
Sign. Level	**	**	**

Means with different superscript letters differ significantly (P≤0.01) **

The control group gives 0.2 ml of D.W, the G1 group gives 50 microgram/kg B.W of SeNPs orally, the G2 group gives 100 microgram /kg B.W of SeNPs orally, the G3 group gives 200 microgram /kg B.W of SeNPs orally, and the G4 group gives 400 microgram/kg B.W orally.

Table 1 shows a clear and significant(P≤0.01) increase in the G3 and G4 groups as compared to the other groups with respect to sperm count in the semen, and no significant (P≤0.01) difference between the G1 and G2 groups as compared to the control group. There is a significant (P ≤ 0.01) increase in the live/dead ratio in all treated groups as compared with the control group. There was a significant (P ≤ 0.01) increase in testicle weight in all treated groups as compared with the control group, and there was a significant (P ≤ 0.01) increase in G2 and G3 groups compared with G1 and G4 groups. No significant (P ≤ 0.01) difference between the G1 and G4 groups, and also no significant difference between the G2 and G3 groups.

4.5 Histological examinations:

4.5.1. Histological examination of control group:

Normal histological architecture of the testicular tissue is demonstrated. Approximately 50% of the examined tissue area shows immature seminiferous tubules (black arrows), characterized by incomplete spermatogenic stratification and relatively narrow lumina. In contrast, the mature seminiferous tubules exhibit an organized germinal epithelium with identifiable stages of spermatogenesis; however, a low density of spermatozoa (yellow arrows) is observed within the tubular lumen. Leydig cells (blue arrows) are evident within the interstitial tissue between seminiferous tubules, displaying eosinophilic cytoplasm and round central nuclei. (Hematoxylin & Eosin)H&E. A: 100x and B: 400x. (Figure 1)

. Histological examination of G1 group:

Normal histological architecture of the testicular tissue is demonstrated. However, approximately 60% of the examined tissue area shows immature seminiferous tubules (black arrows), characterized by incomplete spermatogenic stratification and relatively narrow lumina. In contrast, the mature seminiferous tubules exhibit an organized germinal epithelium with identifiable stages of spermatogenesis; however, a low density of spermatozoa (yellow arrows) is observed within the tubular lumen. (Hematoxylin & Eosin) H&E. A: 100x and B: 400x. (Figure 2)

. Histological examination of the G2 group:

Normal histological architecture of the testicular tissue is demonstrated. The majority of seminiferous tubules (black arrows) appear mature, exhibiting a well-organized germinal layer with clearly identifiable stages of spermatogenesis, including spermatogonia along the basement membrane, primary spermatocytes, and round to elongated

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spermatids progressing toward the tubular lumen. Despite complete spermatogenic layering, a relatively low density of spermatozoa (yellow arrows) is observed within the lumina. Additionally, the thickness of the interstitial tissue (blue arrows) is increased compared with the control and G1 groups, suggesting interstitial expansion consistent with an increased number of Leydig cells. (Hematoxylin & Eosin)H&E. A: 100x and B: 400x. (Figure 3)

4.5.4. Histological examination of G3 group:

A and B/ Normal histological architecture of the testicular tissue is demonstrated. All seminiferous tubules (black arrows) appear mature, exhibiting a well-organized germinal layer with clearly identifiable stages of spermatogenesis, including spermatogonia along the basement membrane, primary spermatocytes, and round to elongated spermatids progressing toward the tubular lumen. The density of spermatozoa within the lumina (yellow arrows) is increased compared with the control, G1, and G2 groups. Additionally, the thickness of the interstitial tissue (blue arrows) is greater than that of the control and G1 groups, indicating interstitial expansion consistent with an increased number of Leydig cells. (Hematoxylin & Eosin) H&E. A: 100x and B: 400x. (Figure 4)

4.5.5 Histological examination of G4 group:

Normal testicular histological building of tissue is demonstrated. All seminiferous tubules (black arrows) are mature and active, showing a well organized germinal layer with different stages of spermatogenesis, including spermatogonia on the basement membrane, primary spermatocytes, and round to elongated spermatids toward the tubule lumen. The lumina contain an increased density of spermatozoa (yellow arrows) compared with the control, G1, and G2 groups. Interstitial tissue (blue arrows) is also thicker than in the control and G1 groups, reflecting expansion associated with an increased number of Leydig cells. (Hematoxylin & Eosin)H&E. A: 100x and B: 400x. (Figure 5)

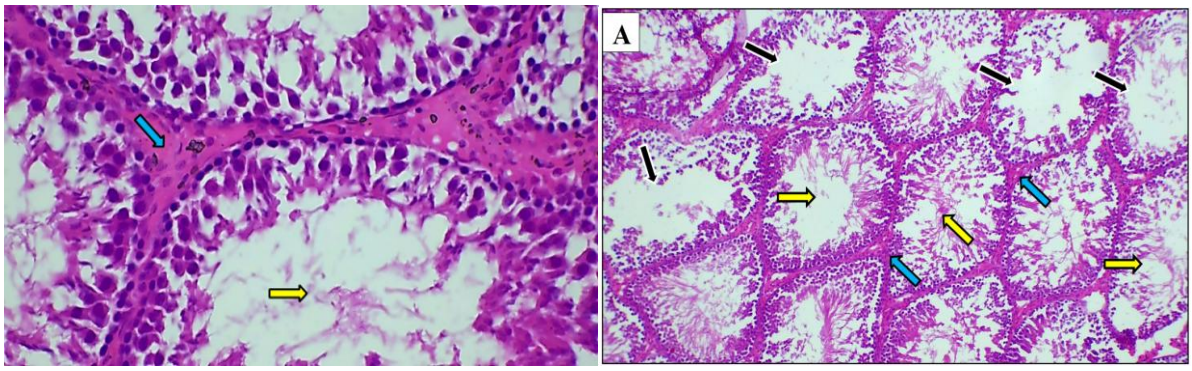

Figure 1: Photomicrograph of the testis of a control group rat.

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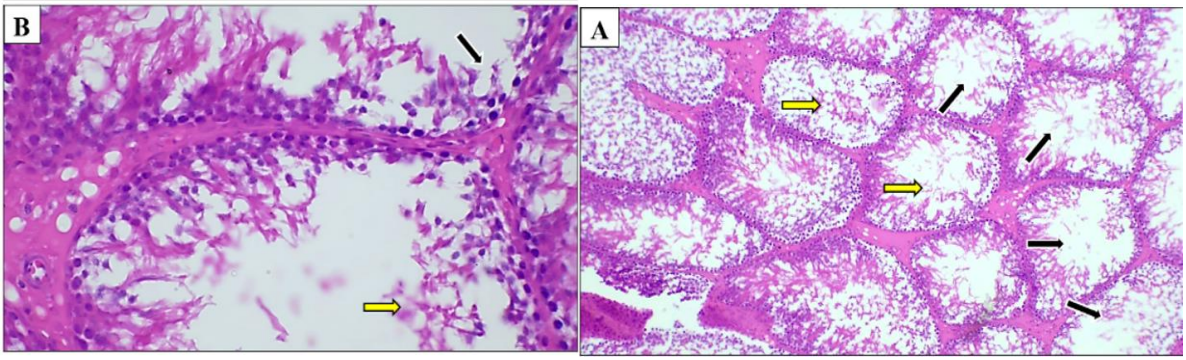

Figure 2: Photomicrograph of the testis of G1 rat.

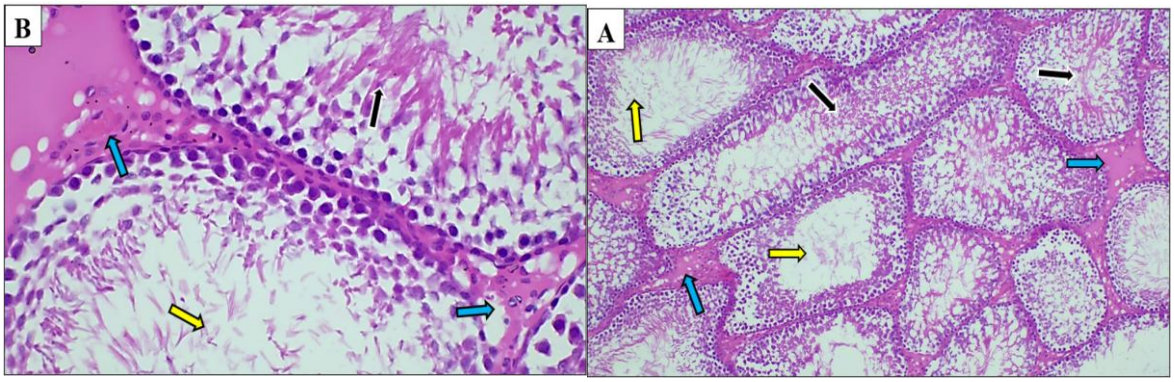

Figure 3 Photomicrograph of the testis of a G2 rat.

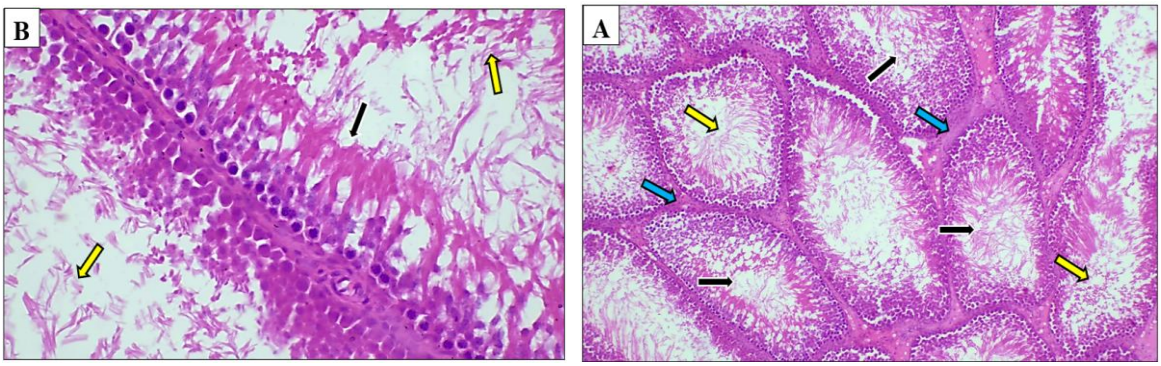

Figure 4 Photomicrograph of the testis of a G3 rat.

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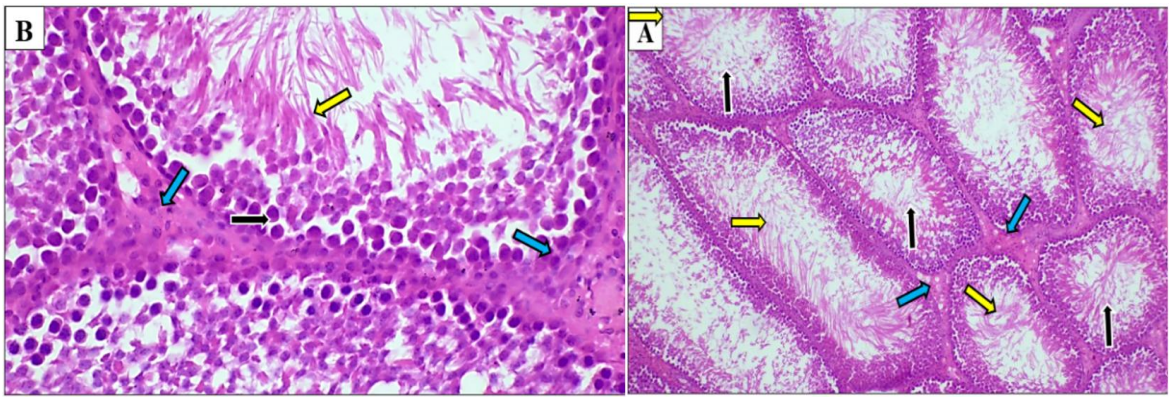

Figure 5 Photomicrograph of the testis of a G4 rat.

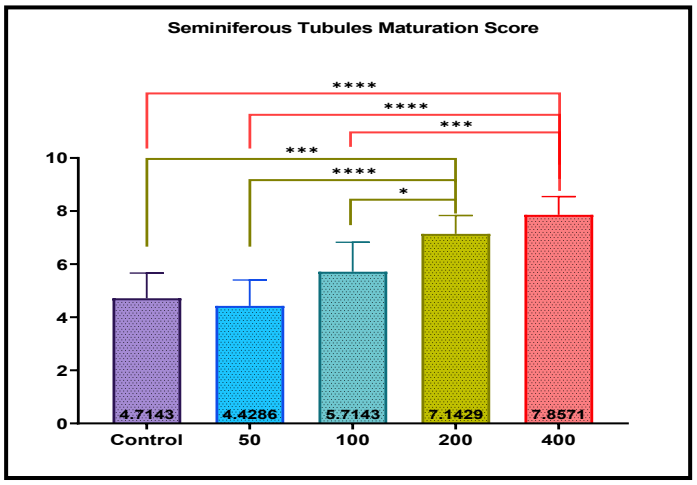

Figure 6 The effect of SeNPs dosage (µg/kg) on the maturation of seminiferous

Tubules, The control group gives 0.2 ml of D.W, the G1 group gives 50 microgram/kg B.W of SeNPs orally, the G2 group gives 100 microgram /kg B.W of SeNPs orally, the G3 group gives 200 microgram /kg B.W of SeNPs orally, and the G4 group gives 400 microgram/kg B.W orally.

Figure 6 shows a significant increase ($p<0.0001$) in the rate of seminiferous tubule maturation in G4 compared to the control and G1 groups. And a significant increase($p<0.001$) in G4 in comparison with the G2 group. And no significant difference between G4 and G3. Also, there is a significant increase ($p<0.0001$) in G3 compared with the G1 group. And significant increase ($p<0.001$) in G3 compared with the G1 group, and a significant increase ($p<0.05$) in the G3 group compared with the G2 group.

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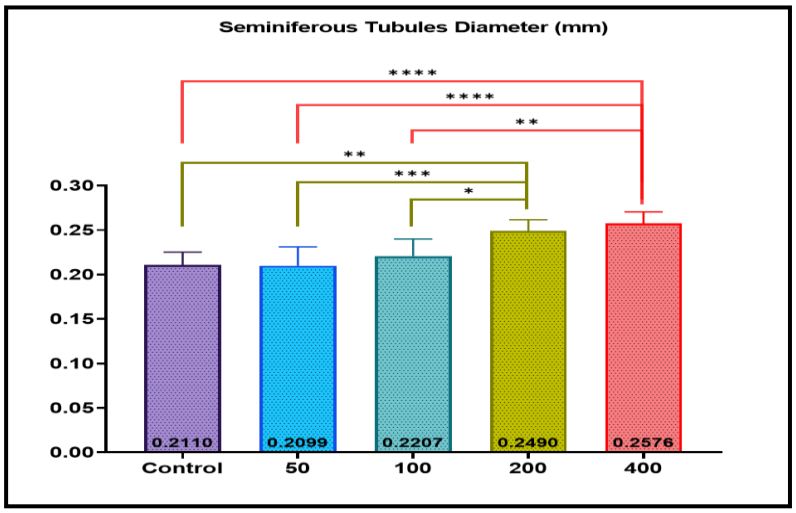

Figure 7: The effect of SeNPs dosage (µg/kg) on Seminiferous tubules diameter (mm), The control group gives 0.2 ml of D.W, the G1 group gives 50 microgram/kg B.W of SeNPs orally, the G2 group gives 100 microgram /kg B.W of SeNPs orally, the G3 group gives 200 microgram /kg B.W of SeNPs orally, and the G4 group gives 400 microgram/kg B.W orally.

Figure 7 shows a significant increase in seminiferous tubule diameter with increasing dose administered to the deferent groups as compared to the control group. There is a significant increase ($p<0.0001$) in G4 compared with the control and G1 groups. and a significant increase ($p<0.01$) in G4 compared with G2 group . There is no significant difference between the G4 and G3 groups. Also, there is a significant increase ($p<0.001$) in G3 compared with the G1 group. and a significant increase ($p<0.01$) in G3 compared with the control group. and a significant increase ($p <0.05$) in G3 compared with G2 group .

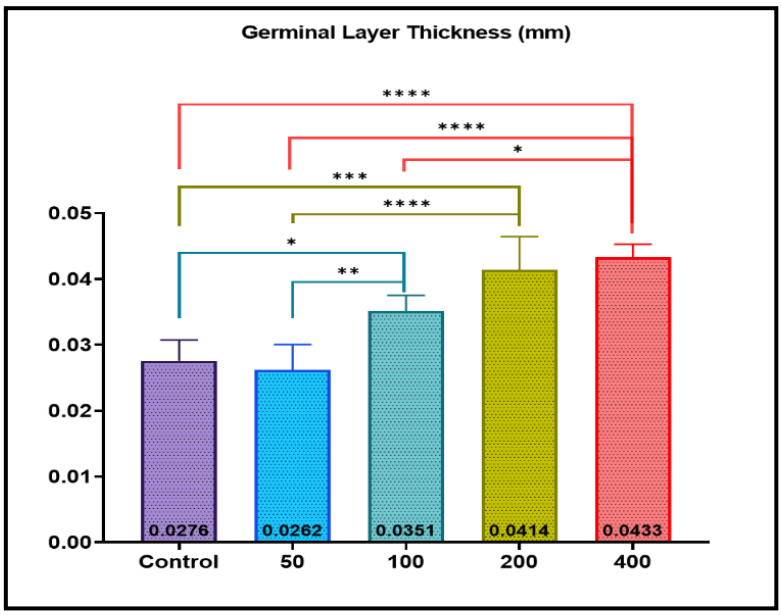

Figure (8) effect of SeNPs dosage (µg/kg) on germinal layer thickness(mm), The control group gives 0.2 ml of D.W, the G1 group gives 50 microgram/kg B.W of SeNPs orally, the G2 group gives 100 microgram /kg B.W of SeNPs orally, the G3 group gives 200 microgram /kg B.W of SeNPs orally, and the G4 group gives 400 microgram/kg B.W orally.

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Figure 8 shows that there is a significant increase ($p<0.0001$) in the germinal layer thickness in G4 compared to the control and G1 groups, and a significant increase ($p<0.01$) in G4 compared with the G2 group. and no significant difference between the G4 with G3 group. Also, there is a significant increase ($p<0.0001$) in G3 compared to the G1 group and a significant increase ($p<0.001$) in the G3 group compared with the control group. in addition there is a significant increase ($p<0.01$) in G2 compared with G1 group and significant increase ($p<0.05$) in G2 compared with control group.

Discussion

The current study showed a significant increase in sperm count, especially in the G3 and G4 groups, compared to the other groups. This is consistent with [22]. The study also showed a significant increase in the percentage of live sperm, Table 1, in all groups compared to the control group. This is consistent with [23], the study also showed a significant increase in testicular weight in all groups compared to the control group. This is consistent with [24], which confirmed that adding nano selenium to feed increases the weight of the testis, stimulates sexual behavior, protects testicular tissue, and improves overall reproductive function. However, current evidence supports a non-linear (bell-shaped or U-shaped) relationship, indicating that both selenium deficiency and excess may adversely affect sperm quality and fertility outcomes [17]. And about the histological examination, this study showed that 50-60% of seminiferous tubules were immature, with low sperm counts and relatively narrow seminiferous ducts in the control and G1 groups. In the group that received 100 µg (G2), the majority of seminiferous tubules were mature, with a very regular germ layer; however, sperm count was low, and interstitial tissue thickness was increased compared to the control and G1 group, which received 50 µg of SeNPs. In the group that received 200 µg, all seminiferous tubules were mature, with a very regular germ layer, clear stages of spermatogenesis, increased sperm count, and increased interstitial tissue volume. The G4 group of the current study showed a significant increase in the maturity and activity of all seminiferous tubules, with a well-organized germinal layer displaying distinct stages of spermatogenesis, including spermatogonia along the basement membrane, primary spermatocytes, and round-to-elongated spermatids toward the tubular lumen. Furthermore, the lumina contain a higher density of spermatozoa compared to the control group. Interstitial tissue is also thicker than in the control group, that agree with [25], and[26].

Conclusion

The study showed a significant and gradual increase in sperm count, live/ dead ratio, and testicular weight. Furthermore, the study demonstrated an improvement in the percentage of mature seminiferous tubules, reaching approximately 100% in the fourth group compared to 50% in the control group, as well as an increase in interstitial tissue thickness, basement membrane thickness, primary spermatocyte density, and greater spermatogenesis. Thus, SeNPs can be considered as an effective dietary supplement for improving male fertility in early developmental stages.

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