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Protective efficacy of *Nerium oleander* extract on spermatogenesis in streptozotocin-induced diabetic rats

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Research Article

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Summary

Men with diabetes frequently experience spermatogenic dysfunction, which is the most significant sign that diabetes has harmed their ability to reproduce. The effect of various doses of the hydro-alcoholic extract of *Nerium oleander* leaves on the pituitary–gonadal axis, sperm motility and number, antioxidant system, changes in testicular tissue structure, and spermatogenesis in healthy and diabetic rats has been examined in the current study. Eighty male rats that had been streptozotocin-induced diabetic and healthy were divided into eight groups: (1) control, (2) *Nerium* (50 mg/kg), (3) *Nerium* (100 mg/kg), (4) *Nerium* (200 mg/kg), (5) DM (6) DM+*Nerium* (50 mg/kg), (7) DM+*Nerium* (100 mg/kg) and (8) DM+*Nerium* (200 mg/kg) and were administered orally for 48 days consecutive. Following the studies, analysis of the testicular tissues' antioxidant capacity as well as sperm parameters, Johnsen's scoring and morphometric evaluation, histology, biochemical and stereology studies were performed.

The outcomes showed that *Nerium* 50 and 100 mg/kg considerably enhanced the testicular morphology, sperm parameters, and reproductive organs to varying degrees in diabetic rats. After *Nerium* 50 mg/kg administration, glutathione peroxidase (GPX) and catalase (CAT) levels in the testicular tissue were increased whereas malondialdehyde (MDA) levels were markedly decreased. *Nerium* may help protect against diabetic-induced spermatogenic dysfunction in male rats by enhancing the activities of antioxidant enzymes in lower dosages.

Introduction

A frequently occurring and well studied consequence of diabetes is spermatogenic dysfunction. The growing body of diabetes research indicates that male diabetic patients are increasingly identified earlier, even as youngsters. This issue is significantly growing every year, spanning more than 30 nations worldwide (Maresch *et al.*, 2018). Sadly, epidemiological studies revealed that ~51% of diabetes individuals have various degrees of reproductive problems (Maiorino *et al.*, 2014). Male diabetic patients have a wide range of typical symptoms, such as a reduction in the weight of the reproductive organs, aberrant spermatogenesis, and sperm abnormalities, as spermatogenic dysfunction is a significant consequence of diabetes (Shi *et al.*, 2017). In males with diabetes, these factors exacerbate and raise the frequency of reproductive harm.

It is difficult to pinpoint the particular pathways causing spermatogenic dysfunction in diabetes. According to earlier research, oxidative stress contributes to spermatogenic dysfunction linked to diabetes (Das *et al.*, 2017; Liu *et al.*, 2017). Reactive oxygen (ROS) is produced in excess when blood sugar levels are high, which may upset the delicate balance between the body's oxidative and antioxidant enzyme systems. As a result, antioxidant enzymes, particularly malondialdehyde (MDA), are unable to prevent the excessive production of lipid peroxide (Agarwal *et al.*, 2014). An earlier investigation verified that diabetic testicular tissues had considerably higher MDA levels and lower levels of antioxidant enzymes (Armagan *et al.*, 2006).

Even though much research has been done on how diabetes affects spermatogenesis, there are presently few clinically effective medications available to address this issue (Han *et al.*, 2019). Therefore, a key problem in the management of diabetic spermatogenic impairment is the search for therapeutic medicines. According to estimates, 1200 plant species are utilized worldwide in traditional and ethnopharmacological techniques to treat diabetes. *Nerium oleander* L. (Apocynaceae) is one such plant (Dey *et al.*, 2015). Previous research on *Nerium indicium*'s anti-diabetic potentials has been limited to measurements of diabetic rats' body weight and glucose load (Sikarwar *et al.*, 2009).

In diabetic rats, *oleander* leaf extracts in chloroform and ethanol were shown to lower blood glucose levels greater than petroleum ether extract. Additionally, the extracts improved oral glucose tolerance while also preventing body weight loss (Bas *et al.*, 2012). The presence of phenolic acids, flavonoids, and methyl phenols in *Nerium oleander* leaves was discovered using HPLC. The main phenolic acids that were found, together with a flavonoid, were gallic acid (GA), 4-hydroxy benzoic acid (4-HBA), vanillic acid (VA), *p*-coumaric acid (PCA), and jasmonic acid (JA; Dey *et al.*, 2015). Different chemical components found in herbal extracts have antioxidant properties. Reactive oxygen species (ROS), which are free radicals that can interact with polyphenols to reduce oxidative stress and subsequently harm cells and tissues, are substances with a high capacity for regeneration (Barku, 2019).

We predicted that *Nerium* would also help with diabetic spermatogenic dysfunction due to the flavonoids' comparable chemical structure and biological effects. To explain how *Nerium* may have protective effects on spermatogenesis in diabetic male rats, the current study postulated that it would increase the activities of antioxidant enzymes.

Materials and methods

Preparation of Nerium oleander leaf extract

Early autumn saw the collection of *Nerium oleander* (family Apocynaceae) leaves from the University of Kerman garden in Iran (35°43′57′′N, 51°28′26′′E; accession no. 286604). The Department of Plant Biology at the Faculty of Biological Sciences accurately recognized the plant (Shiraz University, Shiraz, Iran). The leaves were thoroughly cleaned with double-distilled water, allowed to shade-dry at room temperature for 2 weeks, and then ground into a fine powder. The powder (100 g) was combined with 1000 ml of a 7:1 methanol: water (v/v) solution, then incubated for 18 h at 37°C with 160 rpm of shaking. For 15 min, the mixture was centrifuged (2850 *g*). The pellet was stored in a shaking container with 1000 cc of 70% methanol mixture. A rotary evaporator was used to concentrate the filtrate under reduced pressure after collecting the supernatant from both phases and filtering it. The end product was lyophilized and kept at 20°C until use (Dey *et al.*, 2015).

The studied animals

In the week leading up to the studies, 80 healthy adult male Sprague-Dawley rats (weighing 200–250 g) were acclimated to their surroundings. The animals were housed in conditions of 45–60 % humidity and 20–22°C temperature with 12-h/12-h light/dark cycles. They had unrestricted access to food, water, and conventional mouse chow while living in separate cages. Eight groups of 10 animals each were randomly assigned ($n = 80$):

- Group I: healthy animals were given saline solution 0.2 ml (Control).
- Group II: healthy animals were given 50 mg/kg of *Nerium* (*Nerium* 50).
- Group III: healthy animals were given 100 mg/kg of *Nerium* (*Nerium* 100).
- Group IV: healthy animals were given 200 mg/kg of *Nerium* (*Nerium* 200).
- Group V: animals with diabetes were given normal saline 0.2 ml (DM).
- Group VI: diabetic animals were given 50 mg/kg of *Nerium* (DM+*Nerium* 50).
- Group VII: diabetic animals were given 100 mg/kg of *Nerium* (DM+*Nerium* 100).
- Group VIII: diabetic animals were given 200 mg/kg of *Nerium* (DM+*Nerium* 200).

Streptozotocin (STZ), a single dosage of 60 mg/kg dissolved in NaCl 9% and 100 mM sodium citrate buffer, pH 4.5, was administered intraperitoneally (IP) to rats to cause diabetes. Diabetic blood sugar was defined as 300 mg/dl or greater. For 48 days (according to the spermatogenesis period in rats), gavage was used to treat every animal. The dosages of *Nerium* were chosen in accordance with previous findings of acute toxicity studies (Kumar, 2019), which showed that up to 2 g/kg body weight (BW) oral gavage of the extracts display no clinical and toxicological signs in animals, as well as alleviate haloalkane-induced hepatotoxicity at 200 mg/BW dose (Dey *et al.*, 2016). Based on these arguments, a dosage of 200 mg/BW was chosen for the current trial, which was only 10% as high as the dose of 2 g/kg used for the acute toxicity study (Dey *et al.*, 2019). The animals were killed 24 h after the final treatment, and the testicular tissues from each animal were separated, weighed, and preserved in 10% formalin. Studies on histology, biochemistry, stereology, and Johnsen's scoring and morphometric evaluation were conducted. The Animal Ethics Committee of the institution's criteria followed in conducting this investigation (approval number: IR.ABADANUMS.REC.1398.072).

Analysis of general parameters

The animal's BW was measured on the first and last days, and testicular volume was calculated using water displacement. The animals were decapitated under a mild anaesthetic 48 days following the operation after an overnight fast. The serum was extracted from the blood samples centrifuged from the animal's heart. Testicular tissue preserved in 10% formalin underwent a histological investigation. The additional testicular pieces kept at −80°C were utilized to test the enzyme-linked immunosorbent assay (ELISA) kit for oxidative stress.

Biochemical parameters of serum samples

The serum glucose was measured using a glucometer (EASYGLUCO, Korea) in line with the manufacturer's recommendations. Glucometer strips with the same lot number were used for all of the tests. According to the manufacturer's instructions for the ELISA kit, the levels of the serum hormones follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were tested (SE120089, Sigma, St Louis, MO, USA).

Biochemical parameters of testis tissue

Measurement of glutathione level

The testis tissue homogenate glutathione (GSH) level was assessed using the Ellman technique (Ellman, 1959). Therefore, testis tissue was produced at a ratio of 1:10 and homogenized in ice-cold phosphate-buffered saline (PBS) using a homogenizer. The test buffer (260 µl containing 1 mM EDTA and 0.1 M sodium phosphate at pH 8) and 0.01 M Ellman's reagent (5 µl) were then added along with the tissue homogenate (15 µl). The incubation was carried out for 15 min at room temperature, and the concentration of 5-thionitrobenzoic acid (TNB) was determined at

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a wavelength of 412 nm. A GSH standard curve was used to compare the read optical density (OD) values, and then the GSH concentration in nmol/mg protein was calculated.

Measurement of malondialdehyde content

The primary factor used to quantify oxidative stress is lipid peroxidation. The amount of malondialdehyde (MDA) is a measure of cellular damage (Ohkawa *et al.*, 1979). To ascertain the MDA concentration, 0.25 N hydrochloric acid, 0.375% thiobarbituric acid, and 15% trichloroacetic acid were combined as a working solution. For each sample, the working solution (500 µl) and homogenate (200 µl) were combined in a microtube and immersed in boiling water for 30 min. After cooling, centrifugation at 5000 rpm was carried out for 10 min. The obtained supernatant OD (200 µl) was finally read at 535 nm. The MDA content was calculated in nmol/mg protein.

Measurement of catalase activity level

Catalase activity was measured using the Koroliuk *et al.* (1988) technique. Therefore, tissue homogenate (5 µl) was incubated with H_2O_2 (100 mol/ml) in a Tris–HCl buffer (0.05 mM) at pH 7 for 10 min. The stop solution (50 µl) contained 4% ammonium molybdate. The yellow combination of H_2O_2 and ammonium molybdate was identified at a wavelength of 410 nm. A unit of CAT activity was determined in this test by the amount of enzyme required to break down 1 mol of H_2O_2 per min.

Total antioxidant capacity (TAC)

Total antioxidant capacity was measured in plasma using an enhanced chemiluminescence assay (Kolettis *et al.*, 1999). In this method, ABTS is incubated with peroxidase and H_2O_2 and produces ABTS cationic radical that has a light absorption of 600 nm. The antioxidant in the samples inhibits dye production and, finally, the number of total antioxidants is reported as mM/l.

Histological parameters of testicular tissue

Histological staining

The testicular tissues were fixed in 10% formalin for 24 h, then embedded in paraffin, cut into 5-µm sections, and stained with haematoxylin and eosin (H&E) to look for histological changes such as vacuolization (the appearance of empty spaces in the seminiferous tubules), detachment (the appearance of cohorts of spermatocytes breaking off from the seminiferous epithelium), and sloughing for each blindly read slide, the mean count of six fields was obtained.

Morphometric analysis

After tissue processing using the Motic program (Micro-Optic Industrial Group Co. Ltd, UK), the seminiferous tubule diameter, germinal epithelium height, and seminiferous lumen diameter were all measured. Ten round or nearly round seminiferous tubule cross-sections from each section were randomly selected to measure the diameter of the tubules. Using an Olympus EH light microscope at a magnification of ×40, two perpendicular diameters of each cross-section of the seminiferous tubules were measured, and their means were determined. At the same seminiferous tubules, the height of the germinal epithelium was also measured. In each seminiferous tubule, the tallest and lowest epithelial heights were measured, and their means were then computed.

Ultimately, the seminiferous tubule lumen diameter was determined by deducting the tubule diameter from the epithelium height. Additionally, using a light microscope with an objective lens of ×100, the average numbers of Sertoli cells, spermatogonia, and spermatocytes in 10 seminiferous tubules in stages VII or VIII in each animal were estimated.

Assessment of spermatogenesis

A straightforward approach for assessing spermatogenesis, Johnson's scoring system (Johnsen, 1970) was used to rate the germinal epithelium's maturity. In total, 150 tubules in each mouse were taken into account, and a score from 1 to 10 was assigned to each tubule. Tubules with the most activity (at least five spermatozoa in the lumen) received a score of 10, while those with the lowest activity (total inactivity) received a score of 1.

Sperm motility parameters

To examine sperm motility, spermatozoa were taken from the right epididymis caudal utilizing the computer-assisted semen analysis (CASA) method (Hamilton Thorne, USA). If no movement was seen, the sperm were graded as immotile. Each sample's proportions of sperms that rapidly progressed, slowly progressed, and did not advance were assessed. Using a Neubauer haemocytometer, spermatozoa suspension (from the left epididymis caudal) was made. For morphological evaluations, one drop of the suspension was spread and examined under a light microscope (Goodson *et al.*, 2011; Hajshafiha *et al.*, 2013).

Statistically data analysis

SPSS was used for data analysis (version 21.0, Chicago, IL, USA). The significance of the intergroup difference was assessed using one-way analysis of variance (ANOVA) and Tukey's post hoc test. Additionally, a *P*-value of 0.05 or less was regarded as statistically significant.

Results

Evaluation of fasting blood sugar (FBS)

Figure 1 illustrates the FBS levels of different groups of animals from 7 to 48 days of the experiments; significant reductions were found in the *Nerium* 50, 100 and 200 groups compared with the control group at ($P < 0.003$), ($P < 0.017$) and ($P < 0.006$), respectively. FBS levels were also significantly reduced between DM+*Nerium* at different doses and compared with the DM group ($P < 0.001$).

Effects of Nerium on body and organ weight

According to the findings, there was a significant increase in the initial to final BW of the control group and *Nerium* 50, respectively ($P < 0.001$; $P < 0.015$). There was no significant change in *Nerium* 100 and 200 groups and also in comparison with the control group. A significant reduction was found in the DM group and also showed a significant difference compared with the control group ($P < 0.001$; Figure 1).

There was not only a significant difference in the DM+*Nerium* 50 group, but also a significant difference compared with the *Nerium* 50 group ($P < 0.015$). In the DM+*Nerium* 100 and DM+*Nerium* 200 groups, body and organ weight were reduced significantly ($P < 0.013$, $P < 0.004$; Figure 2).

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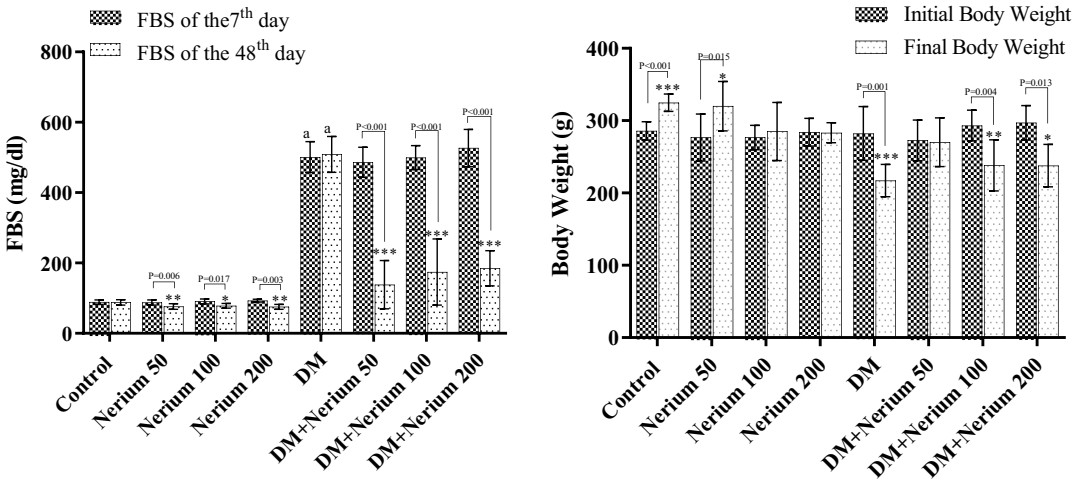

Figure 1. Effect of *Nerium* on fast blood sugar (FBS) level and body weight level in healthy and STZ-diabetic rats. Each value indicates the mean ± standard deviation (SD; $n = 10$). a & * = vs. control group, $^{*}P < 0.05$, $^{**}P < 0.01$; b & # = vs. DM group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

Evaluation of biochemical parameters of serum samples analysis

As seen in Figure 3, testosterone, FSH, and LH hormone levels in the DM group were much lower than those in the control group. In contrast, hormone levels in the DM+*Nerium* 50 group were significantly increased compared with the DM group. There were no discernible differences between the examined groups in all diagrams in which there is at least one related letter. In all figures in which there is no letter in common across the groups, there is a significant difference ($P < 0.05$).

Evaluation of oxidative stress indices

When we compared with the control and healthy groups treated with various dosages, the animal model of STZ for diabetes, made by inducing the disease dramatically, had raised testicular tissue levels of MDA. *Nerium* was administered in modest dosages (50 and 100 groups) and mitigated this rise. When compared with the control group, CAT, GPX, and TAC activities were considerably reduced in the DM and DM+*Nerium* 200 groups. When compared with the DM group, *Nerium* 50 treatment significantly increased CAT, TAC, and GPX activities. Figure 4 reports the effects of diabetes and various *Nerium* dosages on MDA, CAT, TAC, and GPX activities.

Histopathological analysis of testicular tissue

Morphometric analysis

Morphometric analysis is displayed in Figure 5 The numbers of round and long spermatids, Sertoli cells, and Leydig cells were drastically reduced when diabetes was induced. Additionally, following *Nerium* treatment and compared with the control group, seminiferous tubule diameter and germinal epithelium height were reduced, although seminiferous tubule lumen diameter increased. Morphometric parameters were returned to the control value in the DM+*Nerium* 50 group.

Sperm parameters

The *Nerium* 50 and 100 groups showed considerably more motility and sperm production than the control group. In these groups, the percentage abnormality was slightly reduced. Diabetes significantly reduces the amount of sperm. In contrast with the DM group, DM+*Nerium* 50 animals showed a considerable increase in the quantity of sperm. In comparison with the control group, diabetes plus *Nerium* 200 might dramatically reduce sperm motility. Sperm abnormalities were much more prevalent in the DM and high-dose *Nerium* groups. Compared with DM animals, pretreatment with *Nerium* 50 might considerably reduce sperm abnormalities and motility. Table 1 displays the sperm parameter data.

Histopathology analysis

Figure 6 depicts the control group's normal testicular tissue, which includes intact germinal epithelium, seminiferous tubules, and interstitial tissue. *Nerium* 50 spermatogenesis was comparable with that of the control group. There was a substantial difference between the *Nerium* 100 and control groups, but not between the *Nerium* 50 and 100 groups. When compared with the control group and *Nerium* 50, *Nerium* 200 showed a substantial difference, but not when compared with *Nerium* 100. When compared with the control group, there was a considerable difference in the DM group. Germ cell degeneration in varying degrees, disruption of germ cell layers, detachment and sloughing to vacuolization of the seminiferous tubules, and eventually atrophy were all factors.

The DM+*Nerium* 50 group was significantly different from the DM group, while there was no statistical difference compared with the control and no significant changes in histological criteria were observed. The testicular architecture was improved.

In the DM+*Nerium* 100 group, there was a significant difference compared with the control, DM and DM+*Nerium* 50 groups. The testicular tissue structure was severely damaged and atrophy was observed in some seminiferous tubules. The thickness of the basement membrane in the germinal epithelium was increased and interstitial tissue was destroyed. In the DM+*Nerium* 200 group there were no significant differences compared with the DM group, but with other groups, indicating the destruction of testicular tissue.

Discussion

The current investigation demonstrated that the histopathological criteria in the DM, DM+*Nerium* 200, and *Nerium* 200 mg/kg groups were considerably reduced, as were the animal weight,

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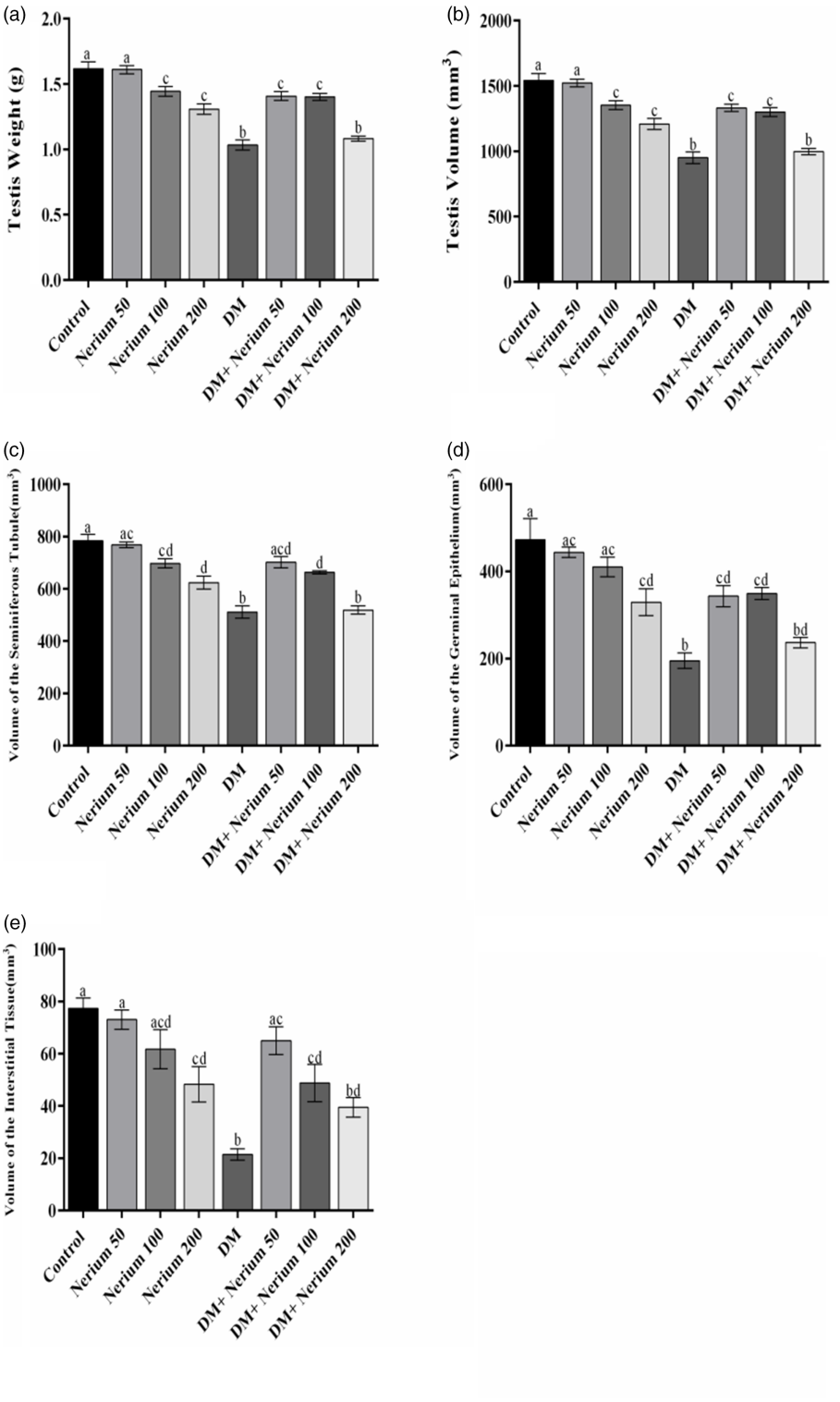

Figure 2. (a–e) Effect of *Nerium* on reproductive organ weight and volume in healthy and STZ-diabetic rats. There were no significant differences between the columns, containing at least one similar letter. However, different letters indicate a significant difference ($P < 0.05$).

testicular volume, testosterone concentration, and sperm quality. *Nerium* 50 and 100 mg/kg exhibited beneficial effects, however, diabetes induction and *Nerium* 200 mg/kg in rats had negative effects. These effects included reduced GPx, TAC, and CAT enzyme activity as well as an increase in MDA in testicular tissue.

As a result of the presence of flavonoid components with antioxidant characteristics, the results demonstrated that, in healthy groups taking *Nerium* at all dosages, the levels of glucose relative to the DM group were much lower. Plant antioxidants stimulate the absorption of glucose into peripheral tissues and have an insulin-like action (Anwar *et al.*, 2021). Antioxidants influence the islets of Langerhans cells, which in turn cause an increase in insulin and a drop in glucose levels in the body (Anastasiou *et al.*, 2021).

Body weight and FBS are affected less by *Nerium* 200 mg/kg than by *Nerium* 50 mg/kg, indicating that *Nerium* is more efficient

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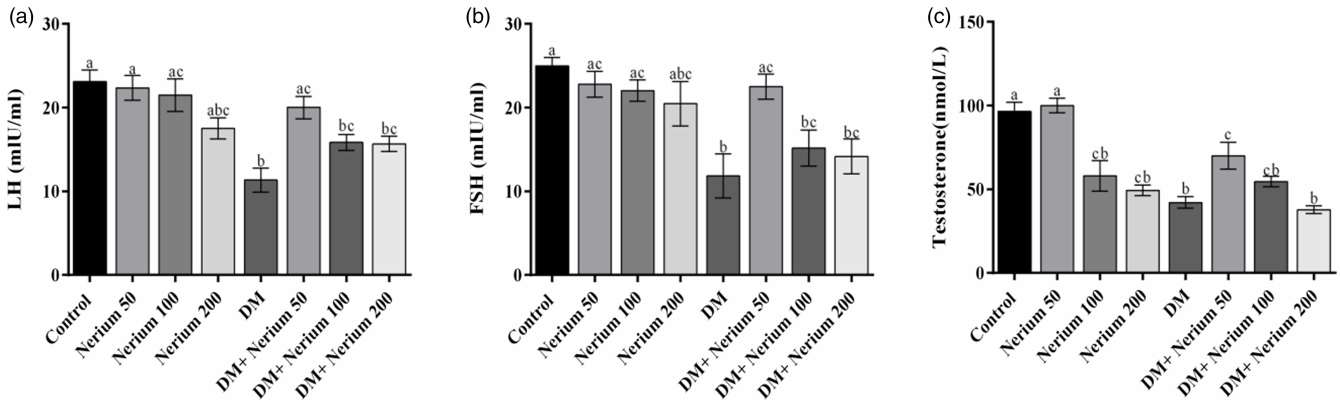

Figure 3. (a–c) Effect of *Nerium* on LH, FSH and testosterone in healthy and STZ-diabetic rats. There were no significant differences between columns containing at least one similar letter. However, different letters reveal a significant difference ($P < 0.05$).

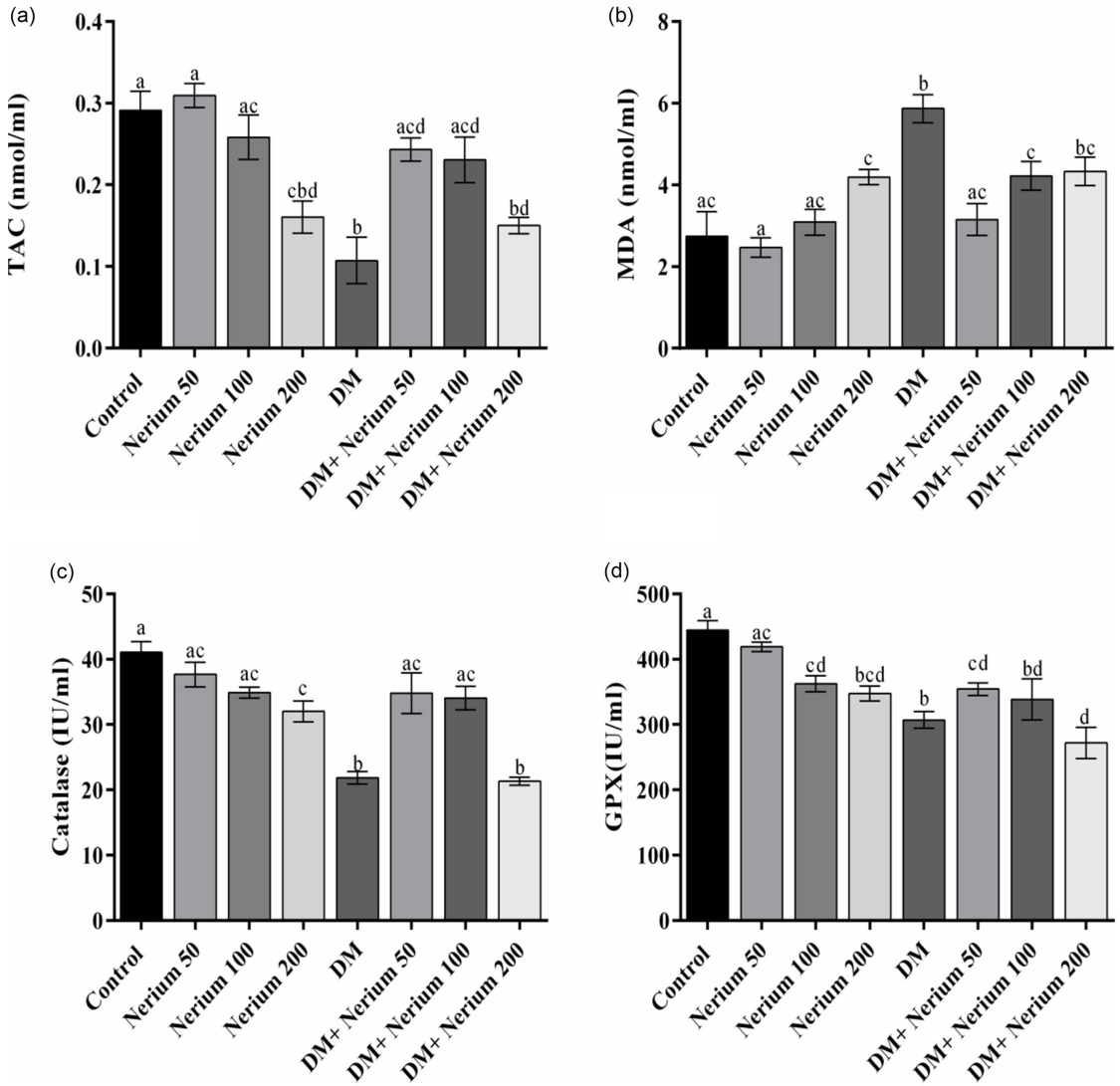

Figure 4. Effect of *Nerium* treatment on oxidative stress-related enzyme production in testis tissue of healthy and diabetic rats. (a) Level of TAC; (b) MDA content; (c) Level of CAT; and (d) GPX level; The data indicate the mean ± standard deviation (SD; $n = 10$). There were no significant differences between columns containing at least one similar letter. However, different letters indicate a significant difference ($P < 0.05$).

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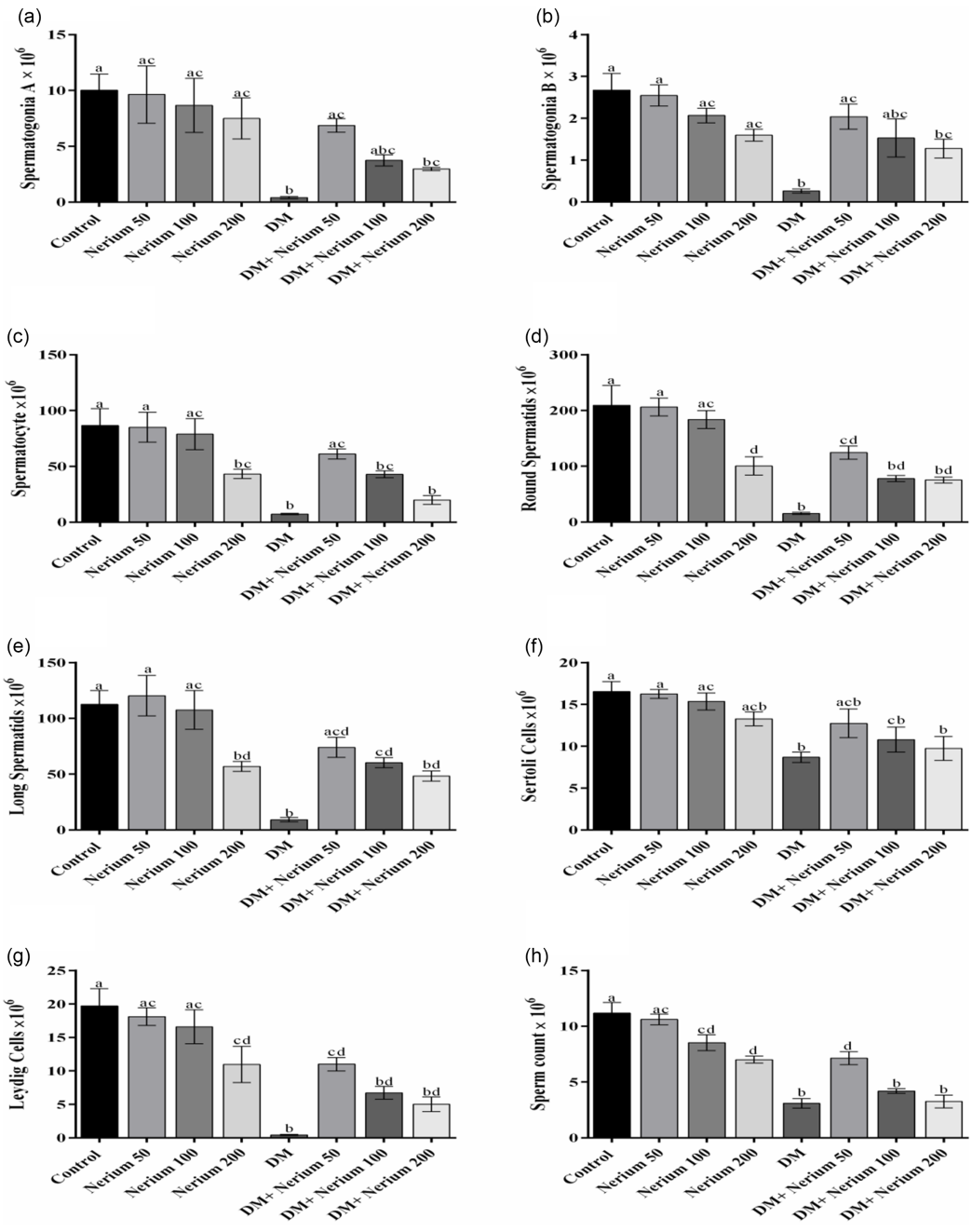

Figure 5. (a–h) Effect of *Nerium* treatment on seminiferous tube parameters in healthy and STZ-induced diabetic rats. Data indicate the mean ± standard deviation (SD; $n = 10$). There were no significant differences between columns containing at least one similar letter. However, different letters indicate a significant difference ($P < 0.05$).

in low than in high doses in diabetic mice. The antioxidant capabilities of flavonoid concentrations may have a determining effect on this result. Flavonoids exhibit stronger antioxidant activities at low concentrations (Rodríguez De Luna *et al.*, 2020). Research suggests that antioxidants might be more effective at lower concentrations due to a phenomenon known as hormesis. Hormesis is the concept that small doses of a substance that are typically toxic at higher levels can actually have beneficial effects on an organism (Bondy, 2023).

Additionally, the *Nerium* compounds contain alkaloids, pectin, polyphenols, and flavonoids that only lower blood sugar levels in diabetic rats produced by STZ and have no effect on healthy rats (Sathyaseelan *et al.*, 2020). As there was no significant difference in serum glucose levels between the healthy group receiving *Nerium* and the control group in the current study, and as *Nerium* only had an effect on hyperglycaemic rats and was unaffected by norm glycaemic rats, no effect of *Nerium* was seen in healthy rats; this is ideal for an anti-diabetic drug. This indicates that *Nerium* reduced blood sugar without boosting insulin secretion and may have done so via other mechanisms.

Additionally, 200 mg/kg of *Nerium* and DM caused a significant reduction in testicular volume and BW. Additionally, *in vivo* research has demonstrated that diabetes causes a reduction in testicular weight. These damaging effects were also noted by histology studies. Testicular weight loss may be primarily caused by atrophy and destruction of testicular tissue (Cheng *et al.*, 2020).

The number of undifferentiated spermatogenic cells present in the testis is largely responsible for its weight. Therefore, a reduction in the number of regenerated cells and suppression of spermatogenesis may be the causes of testicular weight loss. Diabetes may

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Table 1. Sperm parameters

Group	Sperm parameters (mean ± SEM)				
	Progressive sperms	Slow sperms	Non-progressive sperms	Immotile sperms	Normal morphology
Control	63.72 ± 2.67^{a}	4.02 ± 1.01^{a}	20.06 ± 2.51^{a}	12.19 ± 1.19^{a}	93.00 ± 0.89^{a}
Nerium 50	62.34 ± 3.63^{a}	4.59 ± 0.94^{ab}	16.72 ± 1.68^{a}	16.33 ± 3.13^{ac}	91.50 ± 1.23^{a}
Nerium 100	41.22 ± 5.94^{c}	10.69 ± 1.33^{ab}	24.96 ± 5.84^{ab}	23.12 ± 4.27^{abc}	82.33 ± 1.11^{ac}
Nerium 200	20.37 ± 4.75^{bd}	13.63 ± 2.18^{ab}	34.76 ± 3.44^{abc}	31.21 ± 3.67^{bc}	67.5 ± 5.59^{c}
DM	5.89 ± 2.13^{b}	8.93 ± 4.28^{ab}	50.07 ± 5.11^{b}	35.10 ± 6.19^{b}	47.83 ± 6.13^{b}
DM+*Nerium* 50	25.09 ± 5.19^{cd}	15.36 ± 4.39^{ab}	41.68 ± 5.67^{bc}	17.86 ± 1.11^{ac}	70.16 ± 3.33^{c}
DM+*Nerium* 100	8.66 ± 1.20^{bd}	12.50 ± 4.09^{ab}	48.85 ± 5.72^{b}	29.98 ± 2.56^{bc}	65.66 ± 5.35^{c}
DM+*Nerium* 200	5.5 ± 0.95^{b}	17.68 ± 2.56^{b}	42.76 ± 1.46^{bc}	34.04 ± 1.11^{b}	46.33 ± 2.40^{b}

Effect of *Nerium* treatment on the motility of sperm different grades in healthy and STZ-diabetic rats. Data are presented as mean ± standard error of the mean (SEM; $n = 6$). There were no significant differences between columns containing at least one similar letter. However, different letters indicate a significant difference ($P < 0.05$).

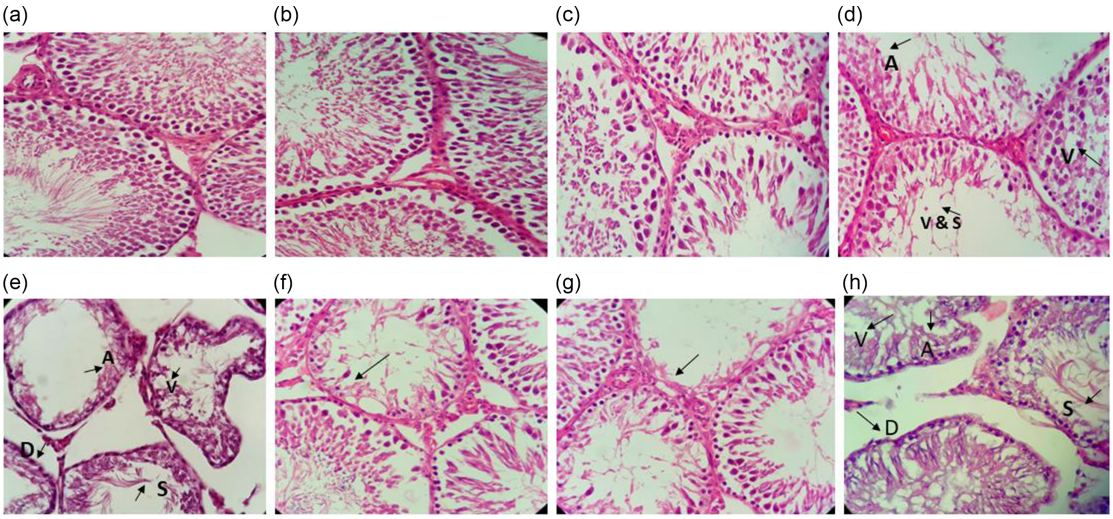

Figure 6. Effect of *Nerium* on histological alterations (H&E staining) in testicular tissue. (a–h) H&E staining. (a) control group; (b) *Nerium* 50 mg/kg group; (c) *Nerium* 100 mg/kg group; (d) *Nerium* 200 mg/kg group; (e) DM group; (f) DM+*Nerium* 50 mg/kg group; (g) DM+*Nerium* 100 mg/kg group; (h) DM+*Nerium* 200 mg/kg group. A: atrophy, V: vacuole, S: sloughing, D: detachment. Magnification ×400 ($n = 6$).

have an effect on the function of Sertoli cells, according to histological abnormalities in the germinal epithelium. According to earlier research, vacuoles are the key morphological indicators of testicular injury and the Sertoli cells' defence mechanisms against infections. (Ghaffari *et al.*, 2019).

When Sertoli cells lose their supportive role, nourishment and hormonal signals intercellular connections are weakened or eliminated, resulting in the loss of germinal cells (Zhao *et al.*, 2023). Another crucial factor in spermatogenesis is the thickness of the basement membrane of the spermatogenesis tubes (Khordad *et al.*, 2020). Diabetes causes the spermatogenesis tube basement membrane to thicken, which inhibits spermatogenesis and, as a result, reduces the size of the seminiferous tubes (Mardanshahi *et al.*, 2019). Histological tests revealed that *Nerium* 50 and 100 mg/kg significantly reduced the thickness of the basement membrane of seminiferous tubules in the DM group. Studies have shown that diabetes significantly lowers sperm parameters such as normal morphology, viability, and motility, which are in line with our findings. (Roshankhah *et al.*, 2019; Forouzandeh and Ghavamizadeh, 2020).

One of the key reasons for diabetes spermatogenic failure is oxidative stress because excessive levels of oxygen radicals in hyperglycaemia cause significant detrimental damage to the sperm membrane, which leads to lipid peroxidation and the production of MDA (Pereira *et al.*, 2021). The most significant cause of spermatogenic dysfunction is lipid peroxidation. Studies revealed that increased MDA in the diabetic group is linked to diabetic testicular injury (Sun *et al.*, 2021). Free radicals can speed up the death of germ cells by apoptosis and decrease the number of germ cells, which eventually results in infertility (Sun *et al.*, 2021). The current study provided evidence that *Nerium* 50 and 100 mg/kg administration in diabetic rats reduced testicular MDA while protecting testicular CAT, GPx, and TAC activities. By changing the number of hormones involved in spermatogenesis, ultra-structural alterations in the seminiferous tube of diabetic rats can interfere with infertility (Maciejewski *et al.*, 2022). Significantly lower testosterone levels were seen in testicular tissue, which caused interstitial tissue damage and seminiferous tubule germinal epithelium degradation (Maciejewski *et al.*, 2022).

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Lower levels of testosterone augmentation in the DM+*Nerium* 50 and 100 mg/kg groups demonstrated that they might lessen the damaging effects of diabetes. According to studies, a reduction in the conversion of progesterone and pregnenolone to testosterone causes the drop in testicular androgens associated with diabetes. Therefore, in addition to having a direct effect on testicular tissue, diabetes can also affect pituitary gonadotropins, which in turn affect the biosynthesis and generation of testosterone (LH and FSH; Rohayem *et al*., 2021).

Figure 4 illustrates that while LH and FSH levels were significantly lower in the DM group compared with the control group, they were not significantly different in the DM+*Nerium* 50 and 100 mg/kg groups compared with the healthy group. This suggests that the DM+*Nerium* 50 and 100 mg/kg groups were successful in restoring normal levels of the pituitary sex hormones that are responsible for testosterone secretion in the testes (Chainy and Sahoo, 2020).

A previous study found that the generation of ROS decreases sperm motility. One of the mechanisms through which diabetes causes alterations in sperm motility is the body's creation of ROS as a result of oxidative stress (Zhu *et al*., 2019). Additionally, diabetes affects the cytoskeleton's structural integrity (Hong *et al*., 2022). It may be found in the sperm tail and is crucial for sperm movement. According to our findings, the doses of 50 and 100 mg/kg were able to significantly reduce the negative effects of diabetes on sperm parameters such as quantity and motility. Studies on the effects of natural products on various models of oxidative stress have been done, and findings were consistent with our study. In the majority of these studies, an agent with antioxidant properties was able to block the action of free radicals, stop cell death, and stop organ dysfunction caused by oxidative stress (Sudharshan *et al*., 2022).

In conclusion, in STZ-induced diabetic rats, *Nerium* attenuated BW, the weight and volume of reproductive organs, sperm parameters, and histological alterations. *Nerium* also increased the activity of antioxidants such as GPx, CAT, and TAC, which lowered the levels of MDA in the testes. The hydro-alcoholic extract of *Nerium* therefore appears to have a protective effect against the harm brought on by diabetes when taken in smaller doses. The supportive effects of this extract against the deteriorating effects of diabetes are also seen in sperm parameters and histological indicators by increasing antioxidant enzyme activities.

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Ethical standard. The authors assert that all procedures contributing to this work complied with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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