Research in Molecular Medicine

Investigating the Protective Effects of Eugenol on Hormonal Levels, Spermatogenesis, and Testicular Tissue Damage in Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus (DM) is a metabolic disorder that can impair male reproductive function by inducing oxidative stress, testicular damage, and hormonal imbalance. Eugenol, a bioactive component of clove, has shown antioxidant and protective effects in various tissues. This study aimed to investigate the effects of eugenol on testicular histology, spermatogenesis, sperm parameters, and reproductive hormone levels in streptozotocin-induced diabetic rats.

Materials and Methods: Thirty-two adult Wistar rats were randomly divided into 4 groups (each with 8 rats): Control, diabetic control, diabetic+eugenol (4 mg/kg/d via gavage for 8 weeks), and eugenol alone. Diabetes was induced with a single intraperitoneal injection of streptozotocin (55 mg/kg). Fasting blood glucose and serum insulin levels of the rats were monitored during the study. At the end of the study, testicular tissues were collected for histopathology and Johnsen scoring. Also, epididymal sperm were analyzed for count, morphology, and motility. Serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were also measured by ELISA.

Results: Diabetic rats exhibited significant hyperglycemia, reduced serum insulin, disrupted testicular architecture, decreased Johnsen scores, reduced seminiferous tubule diameter and epithelial thickness, impaired sperm parameters, and lower reproductive hormone levels ($P<0.05$). Eugenol treatment significantly ameliorated fasting blood glucose; improved insulin levels; restored testicular histology; increased Johnsen scores; and enhanced sperm count, motility, and morphology. Furthermore, serum testosterone, LH, and FSH levels were significantly elevated in the diabetic+eugenol group compared to diabetic controls ($P<0.05$), approaching levels observed in healthy controls.

Conclusion: Eugenol effectively mitigates diabetes-induced testicular damage, improves spermatogenesis, and restores reproductive hormone levels in diabetic rats. These findings suggest that eugenol may have therapeutic potential for protecting male reproductive function under diabetic conditions.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. DM global prevalence continues to rise, and projections indicate that by 2045, more than 700 million adults will be affected [1]. Beyond its well-known systemic complications, including neuropathy, nephropathy, and cardiovascular disease, diabetes induces significant impairment in male reproductive function. Several studies have demonstrated that hyperglycemia and increased oxidative stress lead to testicular dysfunction, hormonal imbalance, impaired spermatogenesis, and reduced fertility in diabetic animals and humans [2]. Oxidative stress is considered a major mediator of diabetes-related complications in the male reproductive system. Chronic hyperglycemia promotes excessive generation of reactive oxygen species (ROS), overwhelms the endogenous antioxidant defense system, and results in oxidative damage to lipids, proteins, and DNA within testicular tissue [3]. Leydig and Sertoli cells are particularly susceptible to oxidative stress, contributing to reductions in testosterone levels, disrupted spermatogenic cycles, and abnormalities in sperm count, motility, and morphology [4]. Histopathologically, diabetic testes commonly exhibit degeneration of seminiferous tubules, vacuolization of Sertoli cells, and germ cell apoptosis [5]. Therefore, therapeutic strategies that counteract oxidative stress and protect testicular architecture may attenuate diabetes-induced reproductive dysfunction. In recent years, natural compounds with antioxidant and anti-inflammatory properties have attracted considerable attention as potential protective agents against diabetic complications.

Eugenol, a phenolic component of clove (Syzygium aromaticum), has been widely studied for its pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic, and cytoprotective effects [6]. Structurally, eugenol contains a free hydroxyl group that readily donates hydrogen atoms, enabling efficient scavenging of free radicals and inhibition of lipid peroxidation. Moreover, eugenol modulates key signaling pathways involved in oxidative stress, such as the Nrf2/ARE pathway, and has been shown to enhance cellular antioxidant enzyme activities [7, 8].

Several preclinical studies have suggested that eugenol may exert beneficial effects on metabolic disorders. For instance, eugenol improves glycemic control, reduces oxidative stress biomarkers, and protects against pancreatic β-cell damage in diabetic models [9]. Its anti-inflammatory properties are mediated through the suppression of cyclooxygenase-2 (COX-2), nuclear factor-κB (NF-κB), and pro-inflammatory cytokines [10].

Given that diabetic testicular damage is strongly associated with oxidative and inflammatory processes, eugenol's multifaceted protective actions make it a promising candidate for mitigating reproductive complications of diabetes. Evidence has also emerged regarding the potential reproductive benefits of eugenol [11]. It has been shown to protect against oxidative testicular injury induced by various toxic agents such as heavy metals and chemotherapeutic drugs [12]. These studies reported improvements in sperm parameters, restoration of testosterone levels, and amelioration of histopathological disruptions in the testes [13]. However, despite these promising findings, there remains a scarcity of comprehensive studies specifically evaluating the protective effects of eugenol on spermatogenesis, reproductive hormones, and testicular structure in diabetic conditions.

Given the significant burden of diabetes-related male infertility and the urgent need for effective protective interventions, further investigation of natural antioxidant compounds is warranted. Therefore, the present study aims to evaluate the protective effects of eugenol on hormonal changes, spermatogenesis, and testicular histopathology in diabetic male rats. Understanding these mechanisms may contribute to the development of novel therapeutic strategies for preserving male reproductive health in diabetic patients.

Materials and Methods

Animals and experimental design

This experimental study was conducted on 32 adult male Wistar rats weighing approximately 200–250 g. The animals were housed under controlled laboratory conditions with a 12-h light/dark cycle, stable temperature (22±2 ˚C), and free access to standard chow and water. After a 1-week acclimatization period, the rats were randomly assigned to 4 groups of 8 animals each. The first group served as the normal control and received only standard food and water throughout the study. The second group functioned as the diabetic control and underwent diabetes induction by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg [14]. The third group consisted of diabetic rats treated with eugenol at 4 mg/kg [11], administered orally by gavage for 8 consecutive weeks. The fourth group included healthy, non-diabetic rats that received the same

dose of eugenol for the same duration. All experimental procedures adhered to ethical regulations for laboratory animal care and use.

Induction of diabetes

To induce experimental diabetes, fasting blood glucose levels were measured in all animals to ensure baseline normoglycemia. STZ was freshly dissolved in cold citrate buffer (0.1 M, pH 4.5) and administered intraperitoneally at 55 mg/kg. Seventy-two hours after injection, fasting blood glucose was measured using a glucometer (EASY GLOCO) by tail-tip incision. Rats showing fasting glucose levels above 250 mg/dL were considered diabetic and included in the diabetic groups. From this point forward, animals in the intervention groups received daily gavage administration of eugenol extract at 4 mg/kg for 56 days, corresponding to the full spermatogenic cycle in rats [11].

Sample collection

At the end of the 8-week treatment period, all rats were anesthetized using a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. Once deep anesthesia was confirmed, blood samples (3–5 mL) were obtained directly from the heart for biochemical and hormonal analyses. Following thoracoabdominal incision, the testes of each animal, along with the epididymides, were carefully dissected under sterile conditions. Both testes were removed and weighed. The left testis was immediately placed in fixative for histological processing. The epididymis was isolated for separate assessments of sperm parameters.

Biochemical measurements

Fasting blood glucose levels were measured 1 week before the induction of diabetes and again 1 week after STZ injection to confirm the establishment of hyperglycemia in the appropriate groups. Measurements were obtained by creating a small incision at the tail tip and analyzing a drop of blood using a standard glucometer. These values were used to monitor the glycemic status of all groups throughout the experiment.

Tissue processing for histological examination

For histopathological evaluation, testicular tissues were fixed in Bouin's solution for 48 hours to preserve cellular structures. Bouin's fixative was prepared using saturated picric acid (75 mL), 37% formalin (25 mL), and glacial acetic acid (5 mL). After fixation, the specimens were rinsed and processed using a standard paraffin-embedding protocol. Dehydration was performed by sequentially immersing the tissues in increasing concentrations of ethanol (50%, 70%, 80%, 90%, 96%, and 2 changes of absolute ethanol), each for 2 hours. Clearing was performed using 3 successive changes of xylene for 1 hour each. Tissues were then infiltrated with molten paraffin at 56 ˚C in 2 stages of 2 hours each, followed by embedding in paraffin blocks using L-shaped metal molds. Once solidified, blocks were trimmed and sectioned at 5 µm thickness using a rotary microtome (Pouyan Teb Khadem, Iran). Sections were floated on a 45 ˚C water bath, mounted onto glass slides, and dried in a 37 ˚C incubator for 12 h to ensure proper adhesion.

Hematoxylin–eosin (H&E) staining

For routine morphological assessment, tissue sections were stained with H&E. Slides were first deparaffinized in xylene and rehydrated through a graded ethanol series down to distilled water. They were then immersed in hematoxylin for 3-5 minutes, rinsed in running water, differentiated briefly in acid alcohol, and blued again under running water. This procedure was followed by staining in eosin for 3 minutes. After staining, the slides were dehydrated through an ascending alcohol series, cleared in xylene, and mounted in Entellan. Under this staining protocol, cell nuclei appeared blue-purple, while cytoplasmic components and extracellular matrix appeared pink. Prepared slides were evaluated microscopically, and representative images were captured for analysis.

Evaluation of sperm parameters

The cauda epididymis was minced in phosphate-buffered saline (PBS) and transferred to a Petri dish containing Ham's medium enriched with 5 µg bovine serum albumin. After incubation at 37 ˚C for 20 min, the resulting sperm suspension was diluted at a ratio of 1:100. A 10-µL drop of the diluted sample was placed on a microscope slide for evaluation of sperm count, morphology, and motility. Spermatozoa were counted using a Neubauer hemocytometer under a light microscope. Morphological assessment considered normal and abnormal head shapes (such as round or elongated heads) and tail abnormalities (including elongated, shortened, or fragmented tails). Sperm motility was classified into progressive (straight), non-progressive (zig-zag or vibrational), and immotile categories. Progressive motility was regarded as physiologically normal, whereas all other patterns were categorized as abnormal.

Assessment of seminiferous tubules (Johnsen score)

To evaluate spermatogenesis, the Johnsen scoring method [15] was applied to 30 randomly selected circular seminiferous tubule cross-sections on each slide by two independent, blinded investigators. The scoring criteria, based on the presence and maturation stage of germ cells, were as follows: a score of 10 indicated complete spermatogenesis with abundant late spermatids; 9, slightly impaired spermatogenesis with many late spermatids; 8, only a few late spermatids; 7, no late spermatids but many spermatocytes; 6, only a few spermatocytes; 5, no spermatocytes but many spermatogonia; 4, only a few spermatogonia; 3, only Sertoli cells; 2, no germ cells and Sertoli cells only; and a score of 1 indicated an entirely atrophic tubule lacking both Sertoli and germ cells. The inter-rater reliability was high, and any discrepancies were re-evaluated to reach a consensus.

Hormonal assays

Collected blood samples were centrifuged at 3500 rpm for 10 min to separate serum. Serum samples were stored at -70 ˚C until measurement. Levels of insulin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were quantified using commercial ELISA kits compatible with rat serum. LH and FSH were measured using kits from ZellBio GmbH (Germany; Cat. No. ZB101825R9648 and ZB10179SR9648), and testosterone was assessed using a kit from Demeditec Diagnostics (Germany; cat. no. 4925-300A). All analyses were conducted according to manufacturers' protocols, and absorbance values were read using a microplate reader.

Statistical analysis

All statistical analyses were performed using SPSS software, version 27.0 (IBM Corp., Armonk, NY, USA). The normality of the data distribution was confirmed using the Shapiro-Wilk test. For comparisons among the four experimental groups, a one-way analysis of variance (ANOVA) was conducted, followed by Tukey's post-hoc test for multiple comparisons using GraphPad Prism software (GraphPad software, version 8.0.2, San Diego, CA, USA). All values are expressed as Mean±SEM. Results with a $P<0.05$ were considered.

Results

Fasting blood glucose and serum insulin levels

At baseline, fasting blood glucose levels in all rats were within the normal physiological range, and no significant differences were observed among the experimental groups ($P>0.05$). One week after the intraperitoneal administration of STZ (55 mg/kg), fasting blood glucose levels increased markedly in diabetic rats compared with the healthy control group ($P<0.05$), confirming the successful induction of diabetes. At the end of the 8-week treatment period, fasting blood glucose remained significantly elevated in the diabetic control group compared with the healthy control ($P<0.05$). However, diabetic rats treated with eugenol exhibited a significant reduction in fasting glucose levels compared with diabetic controls ($P<0.05$), indicating eugenol's hypoglycemic potential. Serum insulin levels followed an inverse pattern: insulin concentration was significantly lower in diabetic rats than in the healthy group ($P<0.05$), whereas eugenol-treated diabetic rats showed a significant improvement in insulin levels relative to diabetic controls ($P<0.05$). No significant differences were observed between healthy controls and the healthy eugenol-treated group (Table 1).

Table 1. Fasting blood glucose and serum insulin levels in experimental groups

Groups	Mean±SEM			
	Fasting Blood Glucose (mg/dL)			Serum Insulin at the End Of Week 8 (ng/mL)
	Diabetes Induction	One Week after STZ Injection	End of Week 8	
Healthy control	102.42±7.25	94.5±5.34	92.4±5.34	1.4±0.16
Diabetic control	98.2±2.6	325.4±4.07*	357.6±15.73*	0.18±0.02*
Diabetic+eugenol	96.3±3.89	305.5±4.03*+	180.2±3.2*+	0.45±0.05*+
Healthy+eugenol	94.25±2.4	92.8±4.7	91.5±4.05	1.42±0.19+

*P<0.05 vs healthy control group, +P<0.05 vs diabetic control group.

Histopathological evaluation

Microscopic examination of H&E-stained sections demonstrated severe degenerative changes in the seminiferous tubules of the diabetic control group. These changes included disorganized germinal epithelium, reduced epithelial thickness, tubular atrophy, basement membrane disruption, and vacuolated or detached germ cells. In contrast, rats in the diabetic group treated with eugenol exhibited notably improved histological features. The seminiferous tubules retained a more regular and circular morphology, the basement membrane appeared continuous, and the organization of germinal cells was largely preserved. Degenerative changes were minimal, and no prominent inflammation or interstitial edema was observed. The seminiferous tubules in the healthy eugenol group were similar to those of the healthy control, showing well-organized germinal layers and intact basement membranes. Representative histological images are shown in Figure 1.

Quantitative analysis showed significant reductions in the Johnsen score, seminiferous tubule diameter, and germinal epithelium thickness in diabetic rats compared with healthy controls ($P<0.05$). Treatment with eugenol significantly improved all three parameters in diabetic rats ($P<0.05$), although the values remained lower than those of healthy controls. No significant differences were observed between the healthy control and healthy eugenol groups, indicating that eugenol does not alter testicular histology in non-diabetic animals. Histological evaluation further highlighted the impact of diabetes and the protective effect of eugenol on testicular structure. The healthy control group exhibited normal testicular architecture, with a high Johnsen score (9.7±0.46), large seminiferous tubule diameter (252.42±4.25 µm), and substantial epithelial thickness (65.5±1.25 µm). In contrast, the diabetic control group showed severe testicular damage, with markedly reduced Johnsen score (4.4±0.17), tubule diameter (145.11±2.57 µm), and epithelial thickness (31.5±2.13 µm). Eugenol treatment in diabetic rats led to significant restoration of testicular morphology, with improvements in Johnsen score (7.25±0.44), tubule diameter (188.5±3.23 µm), and epithelial thickness (52.5±2.15 µm). However, these parameters did not fully reach the levels observed in healthy controls. The healthy eugenol group exhibited values comparable to the healthy control group (Johnsen

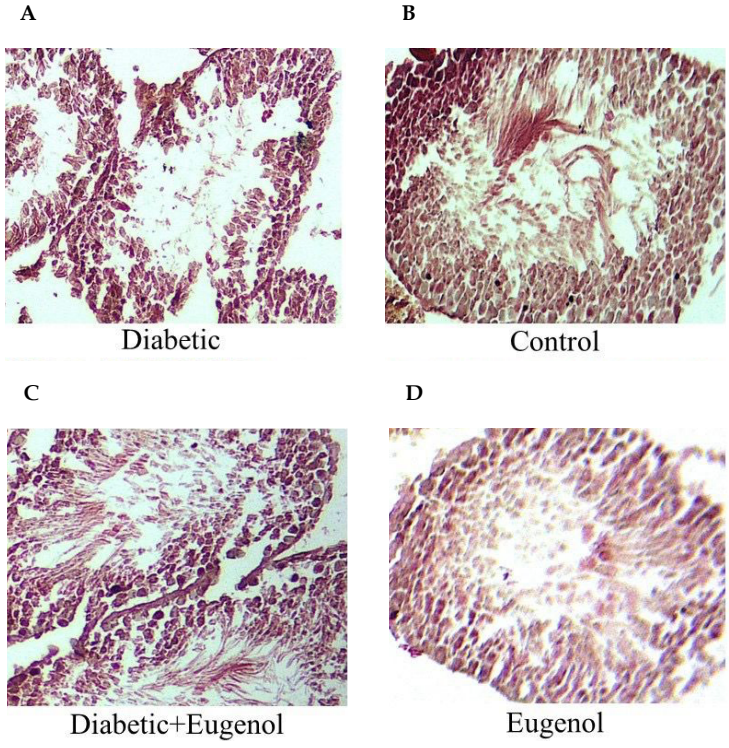

Figure 1. Representative picture of testis histology stained with H&E in different study groups

A) Diabetic group in which severe testicular damage was noted, B) Control group in which normal testicular architecture was seen, C) Diabetic group treated with eugenol, in which there was an improvement in the seminiferous tubule structure (X400 scale bar), D) Eugenol group

Table 2. Counts of Sertoli cells, spermatogonia, primary spermatocytes, and round spermatids in different experimental groups

Group	Mean±SEM			
	Sertoli Cells	Spermatogonia	Primary Spermatocytes	Round Spermatids
Healthy control	30.04±2.17	32.41±1.05	108.41±4.25	210.15±3.85
Diabetic control	17.12±1.65*	21.42±2.05*	62.12±2.6*	97.41±5.07*
Diabetic+eugenol	23.12±2.35*+	25.45±1.02*+	85.5±3.17*+	175.25±2.45*+
Healthy+eugenol	26.14±1.2	31.41±1.35	105.35±3.15	215.35±2.25

*P<0.05 vs healthy control group, +P<0.05 vs diabetic control group.

score: 9.5±0.34; tubule diameter: 255.22±1.7 µm; epithelial thickness: 66.03±1.05 µm), confirming that eugenol administration alone does not significantly affect testicular histology.

Quantification of Sertoli, spermatogonia, primary spermatocytes, and round spermatids

The number of Sertoli cells was significantly reduced in the diabetic control group compared with the healthy control (P<0.05). Diabetic rats treated with eugenol showed a significant increase in Sertoli cell numbers compared with diabetic controls (P<0.05). In contrast, no significant difference was observed between the healthy control and healthy eugenol groups. Similarly, the numbers of spermatogonia, primary spermatocytes, and round spermatids were significantly lower in diabetic controls than in healthy rats (P<0.05). Treatment with eugenol markedly increased the counts of all germ-cell types compared with diabetic controls (P<0.05). The germ-cell counts in the healthy eugenol group were comparable to those in the healthy control group, with no significant alterations observed (Table 2).

Sperm parameters

Sperm count

Sperm count analysis showed a significant decrease in the diabetic control group compared with the healthy control (P<0.05). In contrast, diabetic rats treated with eugenol displayed a significant increase in sperm count compared with diabetic controls (P<0.05). Interestingly, the healthy eugenol-treated group exhibited a slightly higher sperm count than the healthy control, and this increase was statistically significant (P<0.05) (Table 3).

Sperm morphology

Assessment of sperm morphology revealed substantial abnormalities in the diabetic control group. The frequency of abnormal sperm, including elongated heads, headless forms, round-headed sperm, and tail deformities, was significantly higher in diabetic rats than in healthy controls (P<0.05). Treatment with eugenol significantly reduced the proportion of abnormal sperm in diabetic rats (P<0.05), although values did not fully return to those of the healthy group. Sperm morphology in the healthy eugenol group was comparable to the healthy control group, with no significant differences (Figure 2),.

Table 3. Sperm parameters in different experimental groups

Groups	Mean±SEM				
	Sperm Count (×10⁶)	Morphology–normal (%)	Morphology–abnormal (%)	Motility–movable (%)	Motility–non-movable (%)
Healthy control	42.6±3.48	72±1.07	28±1.07	73.6±1.13	26.4±1.13
Diabetic control	12.5±2.59*	20.4±0.5*	79.6±0.5*	12±1.03*	88±1.23*
Diabetic+eugenol	28.2±2.98*+	52±1.17*+	48±1.17*+	47±0.84*+	53±0.84*+
Healthy+eugenol	45.4±5.11*	72.87±0.66	27.13±0.44	75.5±1.11*	24.5±1.12*

*P<0.05 vs healthy control group, +P<0.05 vs diabetic control group.

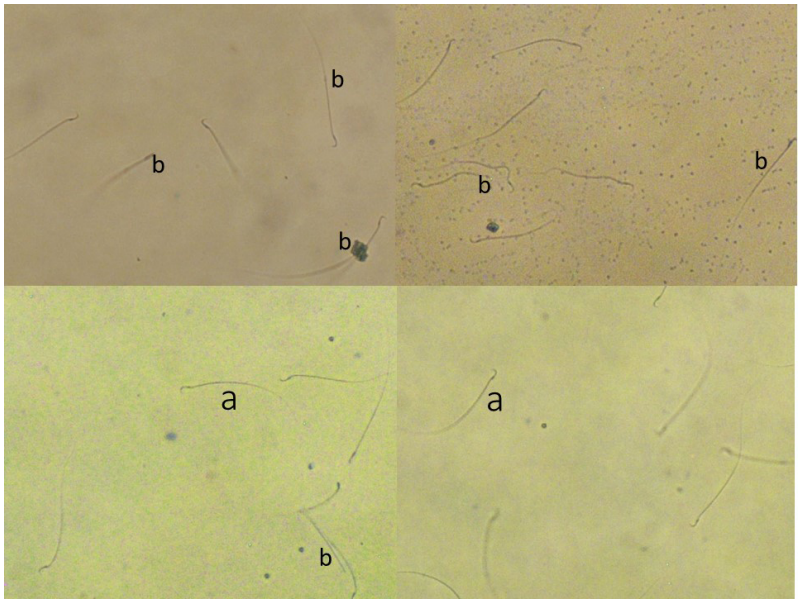

Figure 2. An examination of the morphology of epididymal sperm from mice stained with the diff quick kit
a) Normal sperm morphology, b) Sperm with an abnormal mid-piece

Sperm motility

Sperm motility was significantly impaired in the diabetic control group, which exhibited a marked reduction in motile sperm compared with the healthy control (P<0.05). Treatment with eugenol substantially improved sperm motility in diabetic rats (P<0.05). The healthy eugenol group demonstrated motility values similar to those of the healthy control, with no significant differences, and even showed a slight but significant increase in the proportion of motile sperm compared with controls (P<0.05).

Serum testosterone, LH, and FSH levels

Based on the obtained results, serum testosterone levels were significantly reduced in the diabetic control group (0.62±0.12) compared to the healthy control group (2.35±0.18) (P<0.05). Treatment with eugenol resulted in a significant increase in testosterone (1.65±0.25) in diabetic rats compared to the diabetic control group (P<0.05). However, no significant difference was observed between the healthy control group and the healthy eugenol-treated group (2.54±0.23). Similarly, the levels

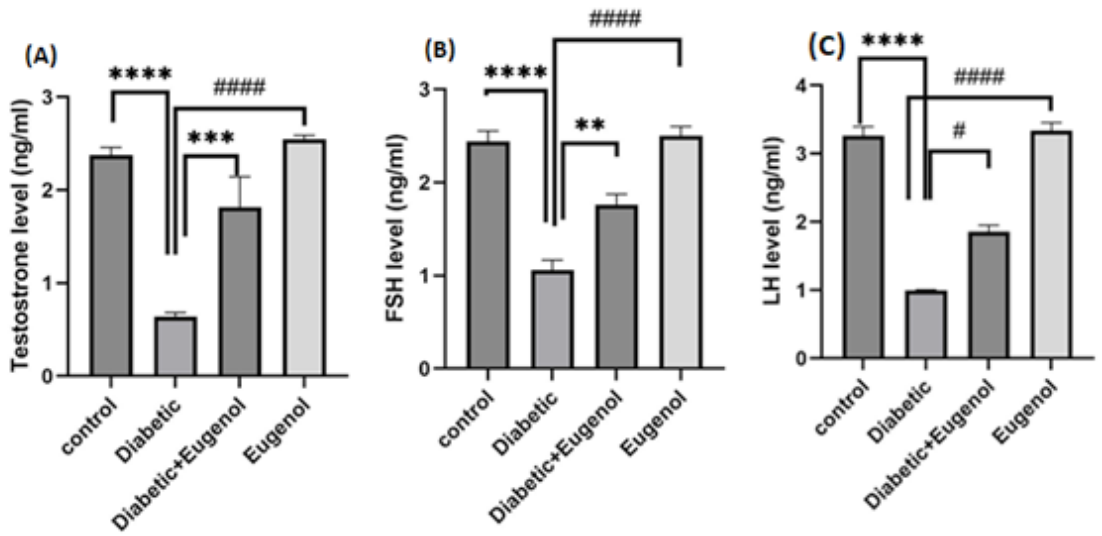

Figure 3. Comparing serum hormone levels in different study groups (n=8 per group)
A) Serum testosterone levels, B) Serum FSH levels (ng/mL), C) Serum LH levels (ng/mL)
*P<0.001, **P<0.01, ****P<0.0001, #P<0.05, ####P<0.0001,

of LH and FSH hormones were also significantly decreased in diabetic rats, with values of (1.09±0.16) and (1.12±0.31), respectively (P<0.05). In contrast, treatment with eugenol led to a significant increase in both hormones compared to the diabetic control group (LH: 1.82±0.21 and FSH: 1.78±0.20) (P<0.05). Finally, hormone levels in the healthy eugenol-treated group (LH: 3.38±0.41 and FSH: 2.5±0.33) showed no significant difference from the healthy control group (LH: 3.25±0.30 and FSH: 2.44±0.15) (Figure 3).

Discussion

The findings of the present study clearly demonstrate that diabetes imposes substantial detrimental effects on testicular structure and function, largely through mechanisms driven by oxidative stress. In the current investigation, diabetic rats exhibited profound reductions in key histomorphometric parameters, including Johnsen score (4.4±0.17 vs 9.7±0.46 in healthy controls), seminiferous tubule diameter (145.11±2.57 vs 252.42±4.25 µm), and germinal epithelium thickness (31.5±2.13 vs 65.5±1.25 µm). These marked declines reflect severe impairment of spermatogenesis and degeneration of the seminiferous epithelium, consistent with prior studies showing that diabetes disrupts seminiferous tubule architecture and depletes germ-cell populations. A substantial body of evidence indicates that oxidative stress is a principal mediator of diabetes-induced testicular damage—elevated ROS and reduced antioxidant capacity trigger lipid peroxidation, membrane destabilization, and cellular injury. Previous studies have consistently reported increased malondialdehyde (MDA) levels in diabetic testicular tissue, linking lipid peroxidation with seminiferous epithelial degeneration [16]. Supporting this mechanism, Shokri et al. (2019) observed reduced tubular diameter, epithelial thinning, and decreased Johnsen scores in diabetic rats due to oxidative stress [17]. Similarly, other investigations have shown that oxidative stress and tissue hypoxia impair germ-cell proliferation and diminish seminiferous tubule diameter [18]. At the same time, another study indicated that hypoxia-mediated ROS formation impairs sperm number, motility, and testicular function [19]. Further supporting the central role of oxidative stress, a previous study demonstrated that resveratrol significantly reduced MDA levels, restored antioxidant enzyme activity, and protected seminiferous tubules against toxic injury [20]. These findings align closely with the protective effects observed in the present study with eugenol, suggesting that phytochemical antioxidants share common mechanisms in stabilizing testicular tissue integrity and improving sperm parameters under oxidative challenge. Hormonal dysregulation also contributes heavily to diabetes-induced reproductive dysfunction. In the present study, diabetic rats showed significantly reduced serum LH, FSH, and testosterone levels, indicating impaired hypothalamic-pituitary-gonadal axis activity. Since LH is crucial for testosterone synthesis via cAMP-mediated pathways and FSH supports Sertoli cell function, reductions in these hormones directly compromise spermatogenesis. These declines are consistent with earlier reports documenting suppressed gonadotropin release and testosterone production in diabetic models [21-23].

Diabetes also significantly impaired sperm parameters, including reduced sperm count and motility, and increased abnormal sperm forms. These changes are consistent with evidence linking oxidative damage and endocrine disruption to defective sperm production [24, 25]. Additionally, antioxidant therapy has been shown to enhance sperm motility, viability, and morphology while decreasing sperm DNA fragmentation, reinforcing the therapeutic potential of antioxidants and supporting the present findings [26]. A major finding of the current study is the substantial ameliorative effect of eugenol on testicular structure and function. Eugenol treatment significantly improved the Johnsen score (7.25±0.44), seminiferous tubule diameter (188.5±3.23 µm), and germinal epithelium thickness (52.5±2.15 µm) compared with diabetic controls. Although not fully restored to normal levels, these improvements highlight eugenol's strong protective capacity. Sperm count, motility, morphology, and serum hormone levels (LH, FSH, testosterone) were also markedly improved. The observed effects are attributable to eugenol's antioxidant, anti-inflammatory, and anti-apoptotic properties, which reduce oxidative damage, support steroidogenesis, and restore cellular integrity. These findings are consistent with those of Saleh et al. who reported enhanced sperm motility and morphology following eugenol treatment [12]. Evidence from studies evaluating combined antioxidant therapies further underscores the efficacy of targeting multiple oxidative pathways simultaneously. A study employing a two-combination antioxidant regimen demonstrated significant improvements in sperm motility, morphology, and membrane integrity compared with controls, paralleling the improvements observed with eugenol in the present study [27]. In addition, chia seed extract has been shown to increase post-thaw sperm motility, viability, and antioxidant gene expression while reducing lipid peroxidation [28], underscoring the broader capacity of plant-derived antioxidants to fortify endogenous antioxidant systems and enhance sperm resilience. Importantly, the healthy eugenol-treated group in the present study

exhibited histomorphometric and hormonal values comparable to those of untreated healthy controls, including a Johnsen score of 9.5±0.34 and a seminiferous tubule diameter of 255.22±1.7 µm. This finding confirms that eugenol does not exert harmful effects on testicular tissue under normal physiological conditions. Despite the promising findings, several limitations must be noted. First, this study used a rat model of diabetes, and although rodent research provides valuable insights, extrapolation to human reproductive physiology requires caution. Future studies should investigate eugenol's efficacy and safety in human subjects, particularly diabetic men with infertility.

Second, although this study evaluated important histological and functional parameters, it did not assess molecular markers such as gene expression related to oxidative stress, apoptosis, steroidogenesis, or inflammation. Such analyses would clarify eugenol's mechanistic actions and allow direct comparison with other antioxidants known to modulate molecular pathways. Third, only one eugenol dose and treatment duration were used; future investigations should explore dose-response relationships, chronic administration, pharmacokinetics, and toxicity thresholds. Fourth, sperm DNA fragmentation, an essential marker of nuclear integrity, was not evaluated. Given that antioxidants can reduce DNA fragmentation, future research should incorporate advanced sperm assays such as SCSA, TUNEL, and COMET. Finally, environmental variables beyond standard laboratory conditions, such as diet or external oxidative challenges, were not controlled. Future studies should evaluate how combined metabolic stressors influence testicular vulnerability and antioxidant responsiveness. Additionally, investigating eugenol's role in post-thaw sperm preservation, as shown for chia seed extract, may broaden its application as an extender additive in reproductive biotechnology.

Conclusion

In conclusion, this study demonstrates that eugenol exerts a potent protective effect against diabetes-induced impairment of male reproductive function. Treatment with eugenol in diabetic rats significantly ameliorated hyperglycemia and improved insulin levels, which was associated with a marked restoration of testicular architecture and spermatogenic efficiency, as evidenced by improved Johnsen scores, seminiferous tubule diameter, and epithelial height. Furthermore, eugenol effectively reversed the detrimental effects of diabetes on sperm quality, enhancing count, motility, and morphology. The significant elevation in serum testosterone, LH, and FSH levels following eugenol treatment indicates its beneficial role in restoring the hypothalamic-pituitary-gonadal axis function compromised by diabetes. These results collectively suggest that the antioxidant and potentially insulinotropic properties of eugenol play a crucial role in mitigating testicular damage, thereby preserving spermatogenesis and hormonal balance. Therefore, eugenol presents a promising therapeutic candidate for protecting male reproductive health in the context of DM.

Ethical Considerations

Compliance with ethical guidelines

The experimental protocol received formal approval from the Ethics Committee of Islamic Azad University (162474155) before commencement

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Authors contribution's

Resources: Nasim Hayati Roodbari and Amir Afshin Khaki; Methodology: Akram Eidi; Supervision: Nasim Hayati Roodbari, Amir Afshin Khaki, and Akram Eidi; Validation and data curation: Amir Afshin Khaki and Akram Eidi; Investigations, data analysis, and writing the original draft: Maryam Shaeghi Rad; Conceptualization, review and editing: Nasim Hayati Roodbari; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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