Title

Targeting TLR4/NF-κB signaling, oxidative stress, and apoptosis by farnesol mitigates cadmium-induced testicular toxicity in rats

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Abstract:

Cadmium (Cd) is a highly toxic heavy metal, and its detrimental effects on reproductive health pose a significant risk to the general population. Farnesol (FAR), a sesquiterpene alcohol, exhibits anti-inflammatory, antioxidant, and anticancer properties. This study investigated the protective effects of FAR against Cd-induced testicular toxicity, focusing on its antioxidant and anti-inflammatory mechanisms. Rats were randomly divided into four experimental groups: control, FAR (10 mg/kg), Cd (1.2 mg/kg), and Cd + FAR. Cd administration caused testicular tissue damage, altered hormone levels, oxidative stress and apoptosis, upregulated TLR4/NF-κB signaling and diminished antioxidants. FAR ameliorated gonadotropins and testosterone, prevented tissue damages, and attenuated oxidative stress. Additionally, FAR significantly attenuated the inflammatory response triggered by Cd, as evidenced by reduced levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and suppression of the TLR4/NF-κB signaling pathway. FAR inhibited testicular apoptosis by upregulating the anti-apoptotic protein Bcl-2 and downregulating the pro-apoptotic markers Bax and caspase-3. These results suggest that FAR mitigates Cd-induced testicular toxicity through cytoglobin upregulation, suppression of TLR4/NF-κB signaling, and inhibition of apoptotic pathways. Thus, FAR represents a promising therapeutic agent for protecting against Cd-induced reproductive damage.

Keywords: Farnesol; Cadmium; Testis; oxidative stress; Inflammation.

1. Introduction

Cadmium (Cd) is a highly toxic heavy metal, and its adverse effects on reproductive health pose a significant risk to the general population [1, 2]. Cd accumulates progressively in vital organs such as the liver, kidneys, testes, and lungs, impairing their physiological functions [3]. This accumulation is attributed to its minimal excretion rate and prolonged biological half-life

[3]. Among these organs, the testes exhibit exceptional susceptibility to Cd exposure due to their high rates of cellular proliferation and metabolic activity, which adversely affect male reproductive function, including testicular integrity and spermatozoa viability [4-7]. Exposure to cadmium (Cd) has the potential to compromise the integrity of the blood-testis barrier, leading to significant adverse effects on testicular function, sperm quality, and overall reproductive health. This disruption can cause structural and functional damage to the testes, impair sperm production and motility, and ultimately reduce fertility, posing serious risks to male reproductive capacity [8, 9]. The primary mechanisms underlying Cd-induced testicular damage include oxidative stress (OS), apoptosis, and inflammation [10-13]. Disruption of cellular homeostasis by Cd leads to a cascade of physiological and pathological alterations, ultimately resulting in testicular dysfunction [14]. A comprehensive understanding of these mechanisms is essential for developing effective strategies to mitigate Cd-induced testicular damage.

Exposure to Cd is closely associated with the generation of reactive oxygen species (ROS). Cd-initiated redox reactions produce hydroxyl radicals, hydrogen peroxide (H_2O_2), and superoxide anions, which can induce genetic alterations linked to oxidative stress, highlighting the pivotal role of oxidative damage in Cd toxicity [15]. Cd induces the generation of ROS through indirect mechanisms, primarily by triggering Fenton-type reactions and other oxidative processes driven by free iron. Moreover, Cd exposure disrupts mitochondrial function, further exacerbating oxidative stress. These combined effects lead to an overproduction of ROS, which can cause cellular damage and impair antioxidant defenses [16]. Spermatozoa are particularly vulnerable to oxidative damage due to their limited DNA repair capacity and high concentration of polyunsaturated fatty acids [17]. Cd-induced ROS generation can activate several signaling molecules, including toll-like receptor 4 (TLR4), leading to upregulation of nuclear factor-kappa B (NF-κB) and subsequent increases in proinflammatory cytokines [18,

19] . Accordingly, Cd exposure has been linked to testicular cell damage, reduced testosterone synthesis, and impaired sexual behavior in piglets [20]. These adverse effects are primarily attributed to the Cd-induced activation of the TLR4/NF-κB signaling pathway. This pathway triggers inflammatory responses and oxidative stress, which disrupt testicular function and negatively impact reproductive behaviors [20]. The findings highlight the detrimental role of Cd in compromising male reproductive health [17]. Oxidative stress not only causes direct structural damage to the testes but its orchestrated work with inflammation promotes apoptosis, as evidenced by elevated expression of pro-apoptotic B-cell lymphoma (Bcl)-2-associated X protein (Bax) and caspase-3, alongside reduced expression of the anti-apoptotic protein Bcl-2 [21, 22]. Therefore, mitigating oxidative stress and inhibiting the activation of the TLR-4/NF-κB signaling could serve as effective therapeutic approaches to protect the testis against Cd toxicity and apoptosis. By reducing oxidative damage and suppressing inflammation, these interventions may help preserve testicular function, maintain normal testosterone production, and confer protection from the harmful effects of Cd exposure.

Natural products have gained significant attention in recent years due to their potential pharmacological benefits, including attenuation of inflammation and oxidative stress [23, 24]. The sesquiterpene farnesol (FAR) is a natural product that has demonstrated significant pharmacological potential in addressing various conditions related to different body organs [25, 26]. This sesquiterpene is naturally present in the essential oils of aromatic plants and is widely used as a flavoring agent in the food industry [27]. Numerous in vivo and preclinical studies have highlighted its diverse properties, including anti-inflammatory, antioxidant, antimicrobial, and antitumor activities [25, 26, 28, 29]. Our earlier research has confirmed the anti-inflammatory and antioxidant effects of FAR in rat models of hypercholesterolemia [26] and pesticide-induced kidney damage [23]. Additionally, studies have shown that FAR can mitigate oxidative stress and protect against hepatocyte injury in mice exposed to

acetaminophen [30]. Despite these well-documented benefits of FAR in various diseases, its potential to counteract Cd reproductive toxicity remains unexplored. This study aims to investigate the protective effects of FAR against Cd-induced testicular injury, emphasizing the involvement of oxidative stress and TLR-4/NF-κB signaling.

2. Materials and Methods

2.1. Animals and treatments

Male Wistar rats weighing 180–210 g were used in this study. The animals were housed in an air-conditioned room with a controlled 12-h light/dark cycle, maintained at a temperature of 22 ± 1 °C and 50-60% humidity, and provided with free access to water and standard diet pellets. The animals were acclimatized to the laboratory conditions for one week prior to the experiment. The rats were then randomly divided into four groups (n = 6). FAR and cadmium chloride ($CdCl_2$) (Sigma, Cas-no. 4602-84-0 and 202908, respectively) were administered in 0.5% carboxymethyl cellulose (CMC) and 0.9% saline, respectively, as vehicles. Group I and groups II received 0.5% CMC and 10 mg/kg FAR [23], respectively, orally for 14 days and intraperitoneal (i.p.) injection of 0.9% saline on day 7. Group III and group IV received 0.5% CMC and 10 mg/kg FAR, respectively, orally for 14 days and 1.2 mg/kg $CdCl_2$ [31] via i.p. injection on day 7 . The dose and route of administration of Cd were selected based on a pilot study and previous investigations studied the toxicity of Cd [31, 32]. The use of 1.2 mg/kg didn't cause mortality and resulted in blood Cd levels range between 1.36 to 1.75 µg/L which falls within the ranges reported in human populations exposed to Cd. For instance, Mortada *et al* have reported 0.8 − 4.5 µg/L blood Cd levels an Egyptian population [33].

Twenty-four hours after the final treatment, rats were anesthetized using ketamine/xylazine, and blood samples were collected via cardiac puncture. The collected blood was allowed to clot and centrifuged to separate serum which was stored at −80 °C until further analysis. The

testes were excised, cleaned, and portions were fixed in 10% neutral-buffered formalin (NBF) while others were homogenized in cold Tris-HCl buffer (10 mM, pH = 7.4).

2.2. Biochemical assays

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were measured using commercially available kits from Elabscience (China, Cat. no. E-EL-R0391, E-EL-R0026, and E-OSEL-R0003, respertively). Malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase were assessed in the testicular supernatants using kits provided by BioDiagnostics (Egypt). The levels of NF-κB p65 (Cusabio, China, Cat. no. CSB-E08788r), interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-alpha (TNF-α) (Elabscience, China, Cat. no. E-EL-R0015, E-EL-R0012, and E-EL-R2856, respectively) were determined using ELISA kits and the absorbance was read on Epoch microplate reader (BioTek, USA). All procedures were conducted in strict adherence to the manufacturers' protocols.

2.3. Histopathological and immunohistochemical examinations

Following fixation for 24 h in 10% NBF, the tissue samples were embedded in paraffin wax, sectioned at 4 μm thickness using a microtome, and stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Sirius red for histological evaluation. The stained sections were examined blindly using Leica QWin DM3000 (Leica, UK). This scoring analysis was determined per cross-sectional area. The most representative six fields were assessed for each section in all groups using 100x magnification via light microscopy transferred to the screen.

For immunohistochemical analysis, 4 μm-thick paraffin-embedded sections were deparaffinized using xylene and rehydrated through a graded ethanol series. Immunoreactivities were evaluated using the Avidin Biotin Peroxidase Complex method as previously described [34]. Antigen retrieval was performed using a citrate buffer (pH 6.0),

followed by blocking of endogenous peroxidase activity with 3% H_2O_2 and non-specific protein binding with 1% bovine serum albumin. Sections were incubated with primary antibodies against cytoglobin, TLR4, cleaved caspase-3 (Biospes, China, Cat. no. YPA1394, YPA2203, and YPA2210, respectively), Bax, and Bcl-2 (Abcam, USA, Cat. no. ab182858) diluted 1:100, followed by HRP-conjugated secondary antibodies. For color development, 3,3'-Diaminobenzidine (DAB) chromogen/substrate kit (ScyTek, USA) was used strictly following the manufacturer's instructions. Stained sections were imaged (6 per rat) and quantified using ImageJ software.

2.4. Statistical Analysis

The normality of the data distribution was assessed using the Shapiro-Wilk test. Data are expressed as mean ± standard error of the mean (SEM) and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Statistical analyses were performed using GraphPad Prism software (version 8). A p-value of <0.05 was considered statistically significant.

3. Results

3.1. FAR ameliorates testicular histopathological alterations induced by Cd

Photomicrographs illustrating the effects of FAR on testicular histopathology are presented in Figure 1. Testicular sections from the control (Fig. 1a) and FAR-only (Fig. 1b) groups exhibited normal histological architecture, characterized by seminiferous tubules with intact basement membranes, well-organized spermatogenic cell layers, and abundant spermatozoa in the lumina. Interstitial tissues displayed typical Leydig cell morphology and structure. In contrast, testicular sections from Cd-intoxicated rats (Fig. 1c) revealed severe histopathological damage, including irregular and thinning seminiferous tubules, disrupted basement membranes, reduced epithelial height of spermatogenic cells, and widespread apoptotic changes (Table 1).

Additionally, edema-induced detachment of spermatogenic cells from the basement membrane was observed, and the tubular lumina were largely devoid of spermatozoa. Interstitial tissues exhibited vascular congestion, edema, vacuolation, and intense basophilic apoptotic Leydig cells. In comparison, testicular sections from rats treated with FAR (Fig. 1d) showed marked improvement, with increased epithelial height of spermatogenic cells, normal tubular structure, and lumina predominantly filled with spermatozoa, although minor vacuolations were observed in some tubules. Interstitial tissues appeared normal, with intact Leydig cells (Table 1).

Testicular sections from the control (Fig. 2a) and FAR-only (Fig. 2b) groups exhibited strong PAS-positive staining in spermatogenic cells. In contrast, Cd-intoxicated rats (Fig. 2c) showed a reduction in PAS staining intensity, whereas FAR treatment (Fig. 2d) resulted in moderate PAS-positive staining. Sirius red staining demonstrated the extent of collagen deposition in testicular tissues. Control (Fig. 3a) and FAR-only (Fig. 3b) groups exhibited minimal collagen fibers along the basement membranes of seminiferous tubules and interstitial tissues. In contrast, Cd-intoxicated rats (Fig. 3c) showed extensive collagen deposition, and FAR treatment (Fig. 3d) reduced collagen fiber accumulation, with moderate staining intensity compared to the Cd group.

3.2. Effect of FAR on gonadotropins and testosterone in Cd-administered rats

The results illustrated in Figure 4 demonstrate a significant reduction in circulating levels of FSH (Fig. 4A), LH (Fig. 4B), and testosterone (Fig. 4C) in rats exposed to Cd compared to the control group ($P<0.001$). Treatment with FAR significantly elevated the serum concentrations of these hormones in Cd-exposed rats ($P<0.001$) with no effect in normal rats.

3.3. FAR mitigates Cd-induced testicular oxidative stress

Cd injection significantly elevated testicular MDA levels while reducing GSH content and the activities of SOD and catalase ($P<0.001$) as depicted in Figure 5A-D. FAR treatment

effectively counteracted these changes, restoring antioxidant levels and reducing MDA ($P<0.001$).

3.4. FAR attenuates TLR-4/NF-κB signaling and inflammation in Cd-exposed rats

TLR-4 expression showed a significant increase in the testes of Cd-exposed rats (Fig. 6A-B) compared to the control group ($P<0.001$). Additionally, levels of NF-κB p65, TNF-α , IL-6, and IL-1β (Fig. 6C-F) were markedly elevated in the testes of Cd-administered rats ($P<0.001$). Treatment with FAR significantly reduced the expression of TLR-4, NF-κB p65, and pro-inflammatory cytokines ($P<0.001$).

3.5. FAR prevents Cd-induced testicular apoptosis

Cd administration significantly increased the expression of pro-apoptotic markers Bax and cleaved caspase-3 while downregulating the anti-apoptotic protein Bcl-2 (Fig. 7A-D; $P<0.001$). FAR treatment reversed these effects, upregulating Bcl-2 and downregulating Bax and caspase-3.

3.6. FAR upregulates testicular cytoglobin in Cd-administered rats

IHC analysis revealed that Cd significantly reduced testicular cytoglobin ($P<0.001$) as depicted in Figure 8. FAR restored cytoglobin levels in the testis of Cd-administered rats ($P<0.001$).

4. Discussion

Exposure to Cd and other heavy metals has been linked to male infertility, as evidenced by studies in rodent models and human epidemiological research [35]. Cd is known to inflict significant damage on various testicular structures, including the seminiferous tubules, Sertoli cells, and the blood-testis barrier. This structural impairment often results in sperm loss and contributes to infertility [35], and studies have recognized oxidative stress and inflammation are as central mechanisms underlying Cd-induced toxicity [13, 36-38]. Furthermore, a direct correlation has been observed between markers of oxidative stress and urinary Cd levels in humans, highlighting the role of oxidative stress in Cd-related reproductive dysfunction [39].

The naturally occurring sesquiterpene alcohol FAR has demonstrated significant pharmacological potential in mitigating various diseases and conditions [25, 26, 28, 29]. Its multifaceted biological activities, including antioxidant, anti-inflammatory, and anti-apoptotic properties, have prompted investigations into its therapeutic potential against Cd-induced testicular injury. This study provides novel insights into the mechanisms underlying the protective effects of FAR against Cd-induced testicular toxicity. Our findings reveal that FAR enhances antioxidant defenses by upregulating cytoglobin, attenuates inflammation by downregulating the TLR4/NF-κB signaling pathway, and inhibits apoptosis by modulating the expression of Bcl-2, Bax, and caspase-3. These results highlight the potential of FAR as a therapeutic agent for preventing Cd-induced testicular damage and suggest new avenues for developing targeted treatment strategies.

Cd exposure is widely recognized for its detrimental effects on male reproductive health, including disruption of the blood-testis barrier, impaired germ cell adhesion, loss of immature germ cells, reduced testosterone levels, and decreased sperm count, ultimately leading to subfertility or infertility [35, 37]. Our findings are consistent with these reports, demonstrating that Cd administration significantly reduced serum levels of FSH, LH, and testosterone. The observed decline in gonadotropin levels highlights the detrimental impact of Cd on the pituitary-gonadal axis, leading to reduced testosterone production [37]. This reduction in testosterone is closely associated with the apoptosis of Leydig cells. Research has consistently shown that Cd exerts damaging effects on key testicular structures, including the seminiferous tubules, blood-testis barrier, and Leydig cells [35]. Within the seminiferous tubules, Sertoli cells (SCs) play a critical role in spermatogenesis by facilitating spermatogonia differentiation and maintaining communication between the tubules and interstitial tissue [40]. Cd exposure in rodents has been shown to cause significant ultrastructural damage to SCs fragmentation of actin filaments [41-43]. Histopathological examination revealed several adverse alterations,

including a reduction in spermatogenic cell populations, apoptotic changes, interstitial edema, fibrosis, congested blood vessels, hemorrhage, inflammatory cell infiltration, and apoptotic Leydig cells in Cd-exposed rats. Consistent with our findings, studies have reported that Cd exposure in rats leads to disorganization of the seminiferous epithelium, a reduction in spermatogonia numbers, and impaired sperm motility, count, and viability [37, 44-46]. Notably, treatment with FAR effectively reversed these hormonal imbalances and ameliorated the testicular tissue structures. This suggests that FAR may protect against Cd-induced endocrine disruption, thereby preserving testicular function. The protective effect of FAR could be directly attributed to mitigation of oxidative stress and inflammation, two key processes mediating Cd toxicity [16, 44-47].

Oxidative stress, characterized by an imbalance between oxidant production and antioxidant defenses, plays a central role in Cd-induced testicular toxicity. Cd exposure generates ROS, which overwhelm cellular antioxidant mechanisms, leading to lipid peroxidation, DNA damage, and cellular dysfunction [48]. In this study, Cd depleted testicular GSH, SOD, and catalase, while increased MDA levels, a marker of lipid peroxidation. These findings align with previous studies showing the involvement of redox imbalance in Cd toxicity. In this regard, numerous studies have highlighted the involvement of oxidative stress in the reproductive toxicity caused by Cd in both experimental animal models and human populations [39, 44-46]. Research conducted on rodents has demonstrated increased levels of lipid peroxidation, oxidative DNA damage, and a decline in antioxidant capacity following Cd exposure [44-46]. Furthermore, a significant positive correlation between urinary Cd levels and markers of oxidative stress, alongside a negative association with semen quality, underscoring the detrimental impact of Cd on male reproductive health [39]. Cd reduces antioxidant defenses by binding to sulfhydryl groups in GSH and interfering with the catalytic activity of key antioxidant enzymes [49-51]. In addition to inducing oxidative stress, excessive ROS triggers

an inflammatory response by activating signaling pathways, including the TLR4/NF-κB axis, which is sensitive to redox changes and promotes the release of TNF-α, IL-6, and IL-1β [18, 19]. These cytokines were increased in the testis of Cd-administered rats in this study. Upregulation of TLR4 and NF-κB have been reported in studies showing the toxic effect of Cd on the heart and nervous system [52, 53]. In combination with ROS, pro-inflammatory cytokines contribute to cell death by causing mitochondrial dysfunction and promoting apoptosis [54]. The disruption of mitochondrial membrane potential and the subsequent release of cytochrome c activate caspase-3, initiating the apoptotic cascade [55]. Consistent with these mechanisms, Cd-induced apoptosis in the testes of rats was confirmed through increased expression of pro-apoptotic markers (Bax and caspase-3) and decreased levels of the anti-apoptotic protein Bcl-2.

Treatment with FAR effectively mitigated oxidative stress and inflammatory response induced by Cd in the testis of rats. FAR decreased MDA, suppressed TLR4/NF-κB signaling and the release of pro-inflammatory cytokines, and enhanced antioxidants in the testis of rats that received Cd. These effects demonstrated the protective role of FAR against oxidative and inflammatory damage induced by Cd. In addition, FAR prevented apoptosis as evidenced by the decrease in Bax and caspase-3 and increased Bcl-2. The beneficial effects of FAR in disease conditions associated with surplus ROS generation and inflammation have been demonstrated in experimental animal models. In our prior research, we have demonstrated that FAR effectively counteracts oxidative stress and inflammation in conditions such as chlorpyrifos pesticide exposure [23] and hypercholesterolemia [26]. In a murine model of asthma, FAR alleviated lung inflammation by suppressing TNF-α levels [56], and reduced lipid peroxidation and inflammation while boosting antioxidant defenses in the lungs of rats exposed to cigarette smoke extract [57]. Moreover, FAR was shown to downregulate TNF-α and IL-6 levels in mice with gliosis [58]. In another study, FAR prevented early tumor formation and oxidative damage

in the kidneys of rats treated with ferric nitrilotriacetate [28]. Our current findings further reinforce evidence supporting the ability of FAR to mitigate oxidative stress and inflammation effectively. Furthermore, our study provided information on the involvement of cytoglobin upregulation in the protective mechanism of FAR against Cd testicular toxicity. Cytoglobin, a member of the globin family, has recently emerged as a key regulator of oxidative stress and cellular signaling. It scavenges ROS and modulates apoptotic pathways, thereby protecting cells from oxidative damage [59]. Research has shown that the suppression of cytoglobin contributes to cellular damage by inducing oxidative DNA damage, while its upregulation reduces ROS and protects against cell death [59, 60]. Notably, cytoglobin exhibits SOD-like activity, effectively inhibiting the formation of superoxide radicals and peroxynitrite [61]. Additionally, cells and tissues deficient in cytoglobin were found to be more prone to fibrotic changes and inflammatory responses following radiation exposure [59]. In our study, Cd administration significantly downregulated cytoglobin expression, while FAR treatment restored its levels. This suggests that the antioxidant and anti-apoptotic effects of FAR may be mediated, in part, through upregulation of cytoglobin.

While this study provides valuable insights into the protective effects of FAR against Cd-induced testicular toxicity, several limitations should be acknowledged. First, the study was conducted in a rodent model, which may not fully replicate the complexity of human reproductive physiology and Cd exposure scenarios. Second, the study focused on the acute toxicity of Cd. The long-term consequences of Cd exposure and the sustained efficacy of FAR in mitigating testicular damage remain to be investigated. Third, although the study explored key mechanisms such as oxidative stress, inflammation, and apoptosis, other potential pathways, such as epigenetic modifications or hormonal signaling cascades, were not examined. Future studies could explore these additional mechanisms to provide a more comprehensive understanding of Cd toxicity and the protective effects of FAR. Fourth, the

dose-response relationship of FAR and its effect on sperm parameters were not thoroughly evaluated. Determining the optimal therapeutic dose of FAR for maximum efficacy and minimal side effects would be essential for its potential clinical application. Addressing these limitations in future research will strengthen the findings and enhance the translational potential of FAR as a therapeutic agent for Cd-induced reproductive toxicity.

5. Conclusion

This study highlights the therapeutic potential of FAR in preventing Cd-induced testicular injury. FAR exerts its protective effects through multiple mechanisms, including upregulation of cytoglobin to enhance antioxidant defenses, downregulation of the TLR4/NF-κB pathway to attenuate inflammation, and modulation of apoptotic signaling to inhibit germ cell loss. By restoring hormonal balance, reducing oxidative stress, and suppressing inflammatory and apoptotic pathways, FAR effectively mitigates Cd-induced testicular damage. These findings underscore the potential of FAR as an adjuvant therapy for individuals exposed to environmental Cd toxicity. Future studies are needed to explore the clinical applicability of FAR and its potential synergies with other therapeutic agents to further enhance its protective effects.

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Declaration of Competing Interest

All authors declare no conflict of interests in relation to the manuscript.

Ethics declarations:

All animal experiments comply with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8523, revised 1996). The study was approved by the ethics committee of Al-Azhar University (AZ-AS/PH-REC/45/2024).

Availability of data and materials

The manuscript contains all data supporting the reported results.

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Tables:

Table 1. Histopathological lesions scoring.

	Control	FAR	Cd	FAR + Cd
Degenerated seminiferous tubules	0.17 ± 0.16	0.17 ± 0.16	3.83 ± 0.19***	1.83 ± 0.30$^{***\#\#\#}$
Epithelial height of spermatogenic cells	4.0 ± 0.00	4.0 ± 0.00	0.83 ± 0.32***	2.33 ± 0.28$^{***\#\#\#}$
Spermatozoa	3.83 ± 0.17	3.67 ± 0.20	0.34 ± 0.22***	2.50 ± 0.67$^{\#\#}$
Apoptotic Leydig cells	0.33 ± 0.21	0.33 ± 0.21	3.82 ± 0.16***	1.67 ± 0.31$^{\#\#\#}$
Apoptotic spermatogenic cells	0.33 ± 0.21	0.33 ± 0.21	3.83 ± 0.16***	2.00 ± 0.36$^{***\#\#\#}$
Detached spermatogenic cells	0.17 ± 0.16	0.17 ± 0.16	3.62 ± 0.30***	0.83 ± 0.30$^{\#\#\#}$
Congested blood vessels	0.17 ± 0.16	0.33 ± 0.21	3.50 ± 0.34***	0.67 ± 0.33$^{\#\#\#}$

Data are mean ± SEM. ***P<0.001 versus Control. $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 versus Cd.

Figures

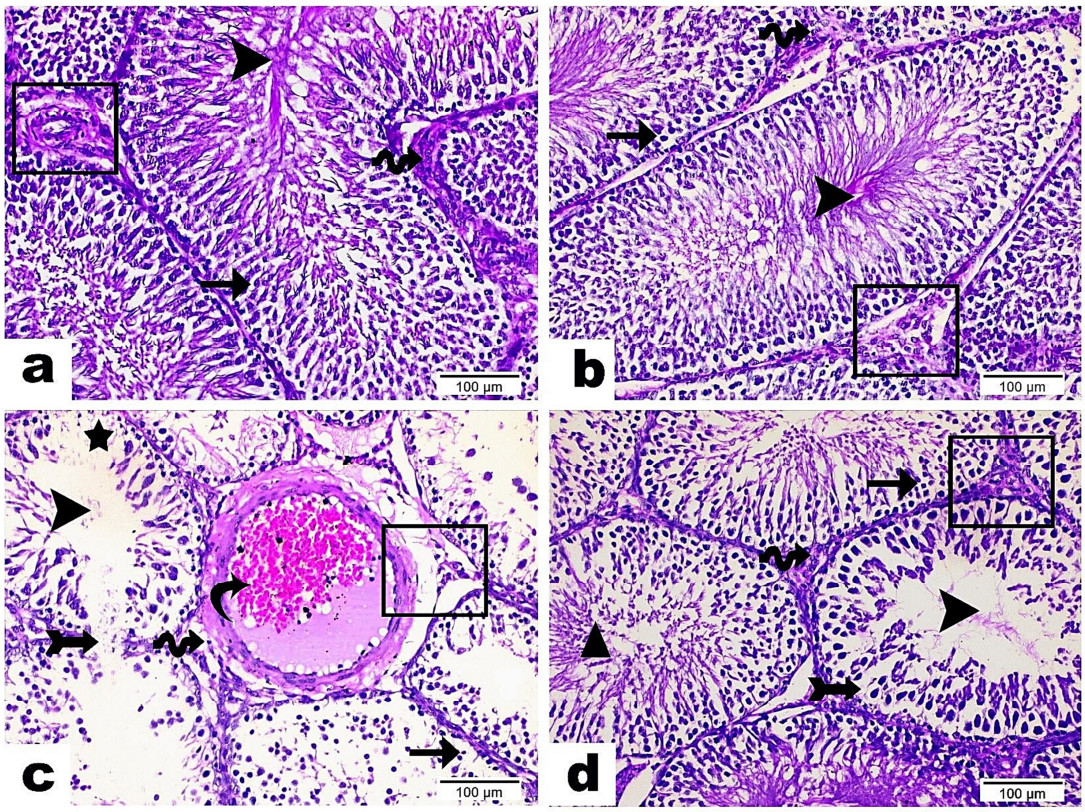

Figure 1. Photomicrographs of H&E-stained sections showing the protective role of FAR against Cd-induced histopathological alterations of testicular tissues. **(a-b)** Testis section from the control (a) and FAR-supplemented (b) rats showing normal seminiferous tubules with lining of spermatogenic cells (arrow) resting on intact basement membrane and engaged with many sperms in its lumen (arrowhead), and interstitial tissue (rectangle) with Leydig cells (wave arrow), **(c)** Cd-administered rats showing irregular and thin seminiferous tubules with damaged basement membrane (arrow with tail), decrease in spermatogenic cells (star), apoptotic changes in most of the spermatogenic cells, edema leading to detachment of spermatogenic cells from the basement membrane (arrow), tubular lumen appeared empty from spermatozoa (arrowhead), congested blood vessels (curvy arrow), edema, vacuolation (rectangle), and intense basophilic apoptotic Leydig cells (wave arrow), and **(d)** Cd-administered rats treated with FAR showing improvement in spermatogenic cells (arrow) except few tubules developed with vacuolations among spermatogenic cells (arrow with tail),increased number of sperms (triangle) although little tubules appeared empty (arrowhead), regular structure of interstitial tissue (rectangle) with intact Leydig cells (wave arrow). (x200, Scale bar = 100 µm).

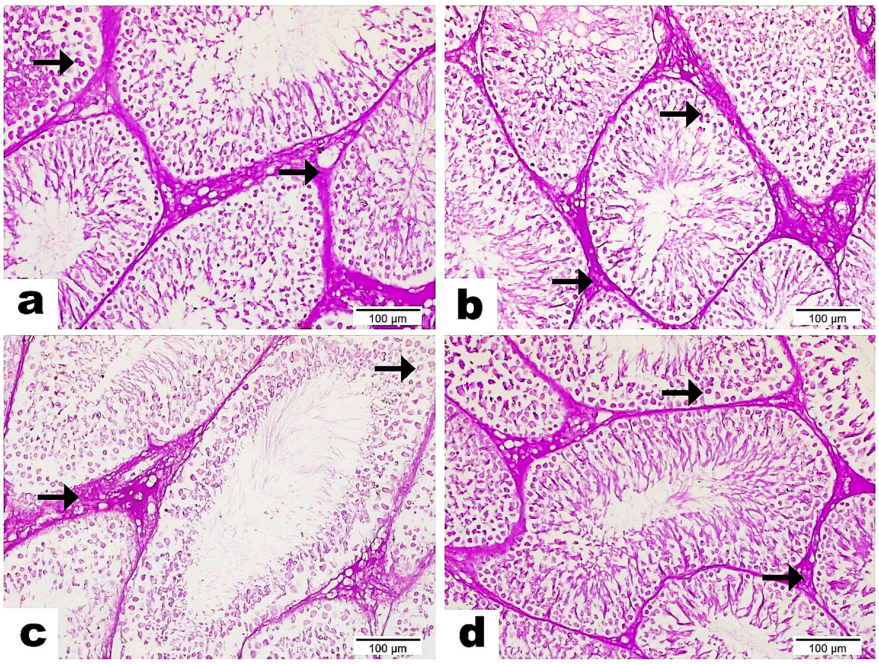

Figure 2. Photomicrographs of PAS-stained sections in the testis of **(a-b)** control (a) and FAR-supplemented (b) rats showing strong positive PAS staining and intact interstitial tissue (arrows), **(c)** Cd-intoxicated rats showing a distinct reduction in the intensity of PAS staining (arrows), and **(d)** Cd-intoxicated rats treated with FAR showing increased PAS staining intensity (arrows). (x200, Scale bar = 100 µm).

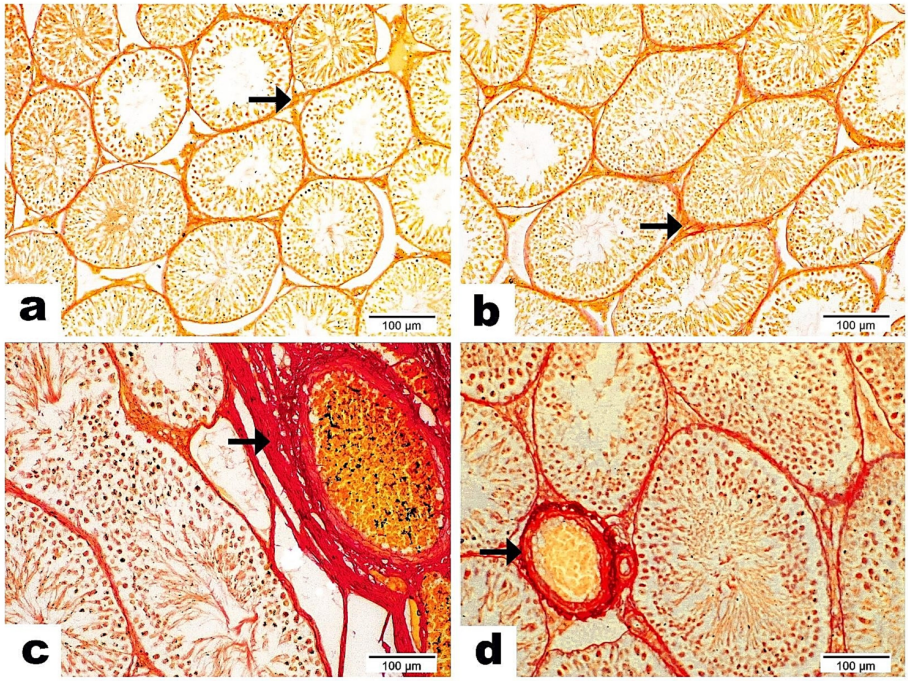

Figure 3. Photomicrographs of Sirius red-stained sections in the testis of **(a-b)** control (a) and FAR-supplemented (b) rats showing a little quantity of collagen fiber along the basement membrane of seminiferous tubules and interstitial tissue (arrow), **(c)** Cd-intoxicated rats showing increased collagen fibers organized as interstitial fibrosis (arrow), and **(d)** Cd-intoxicated rats treated with FAR showing a noticeable reduction in collagen fiber (arrow). (x200, Scale bar = 100 µm).

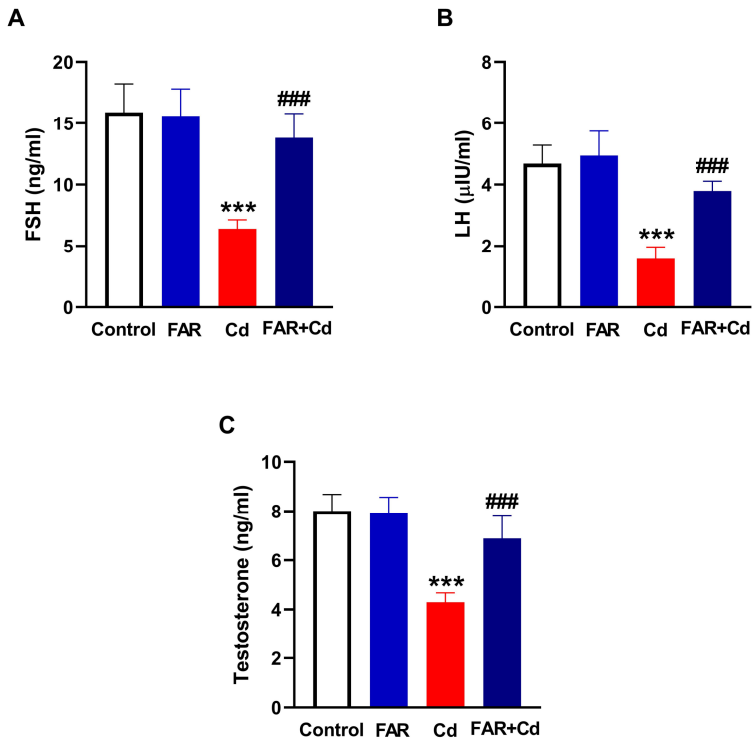

Figure 4. FAR alleviated gonadotropins and testosterone in Cd-administered rats. FAR increased in serum FSH (A), LH (B), and testosterone (C). Data are mean ± SEM, (n = 6). ***P<0.001 versus Control. $^{###}$P<0.001 versus Cd.

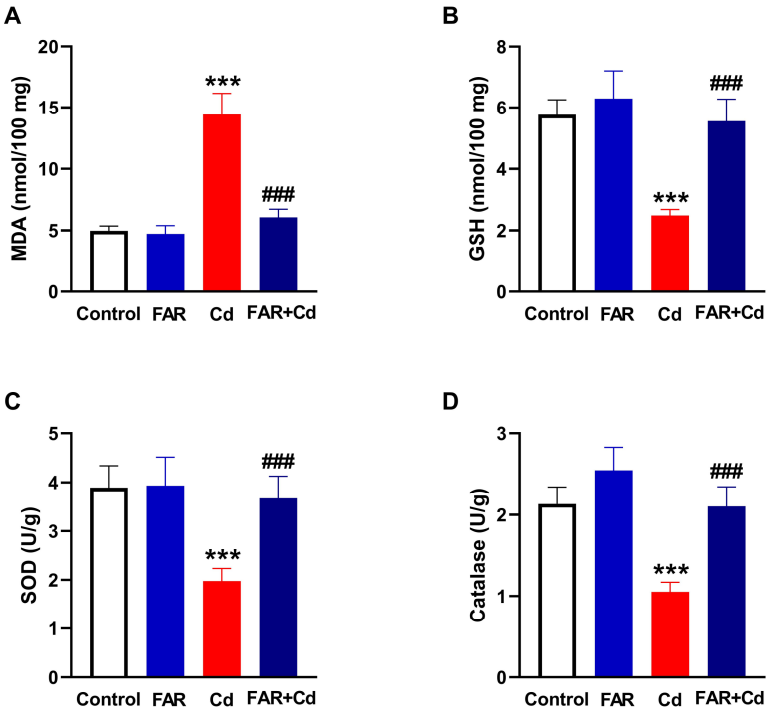

Figure 5. FAR mitigated Cd-induced testicular oxidative stress. Treatment with FAR decreased MDA (A), and increased GSH (B), SOD (C), and CAT (D). Data are mean ± SEM, (n = 6). ***P<0.001 versus Control. $^{###}$P<0.001 versus Cd.

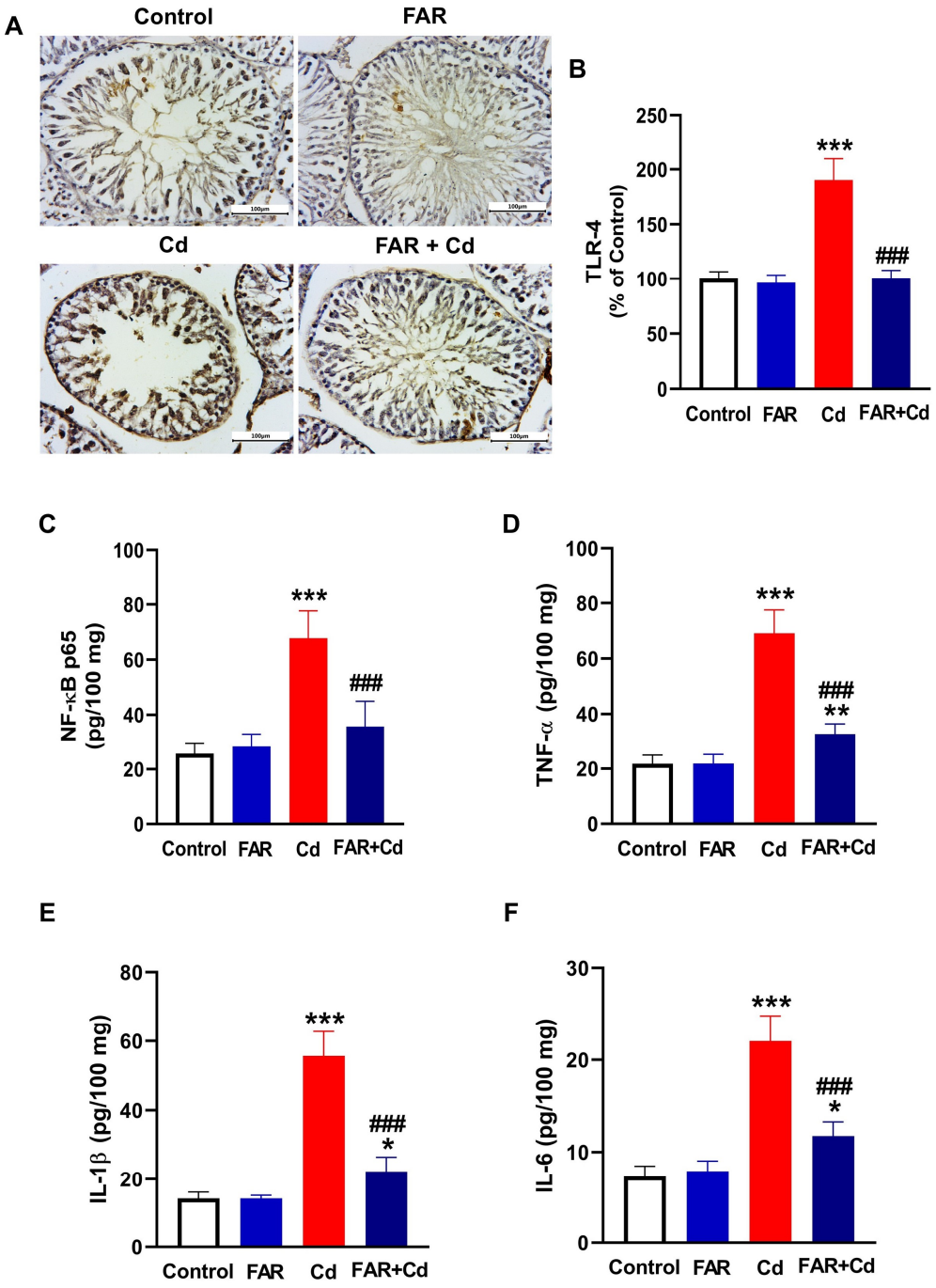

Figure 6. FAR attenuated inflammation in Cd-administered rats. FAR downregulated the expression of TLR4 (A-B) and NF-κB p65 (C), TNF-α (D), IL-1β (E), and IL-6 (F). Data are mean ± SEM, (n = 6). *P<0.05, **P<0.01, and ***P<0.001 versus Control. $^{###}$P<0.001 versus Cd.

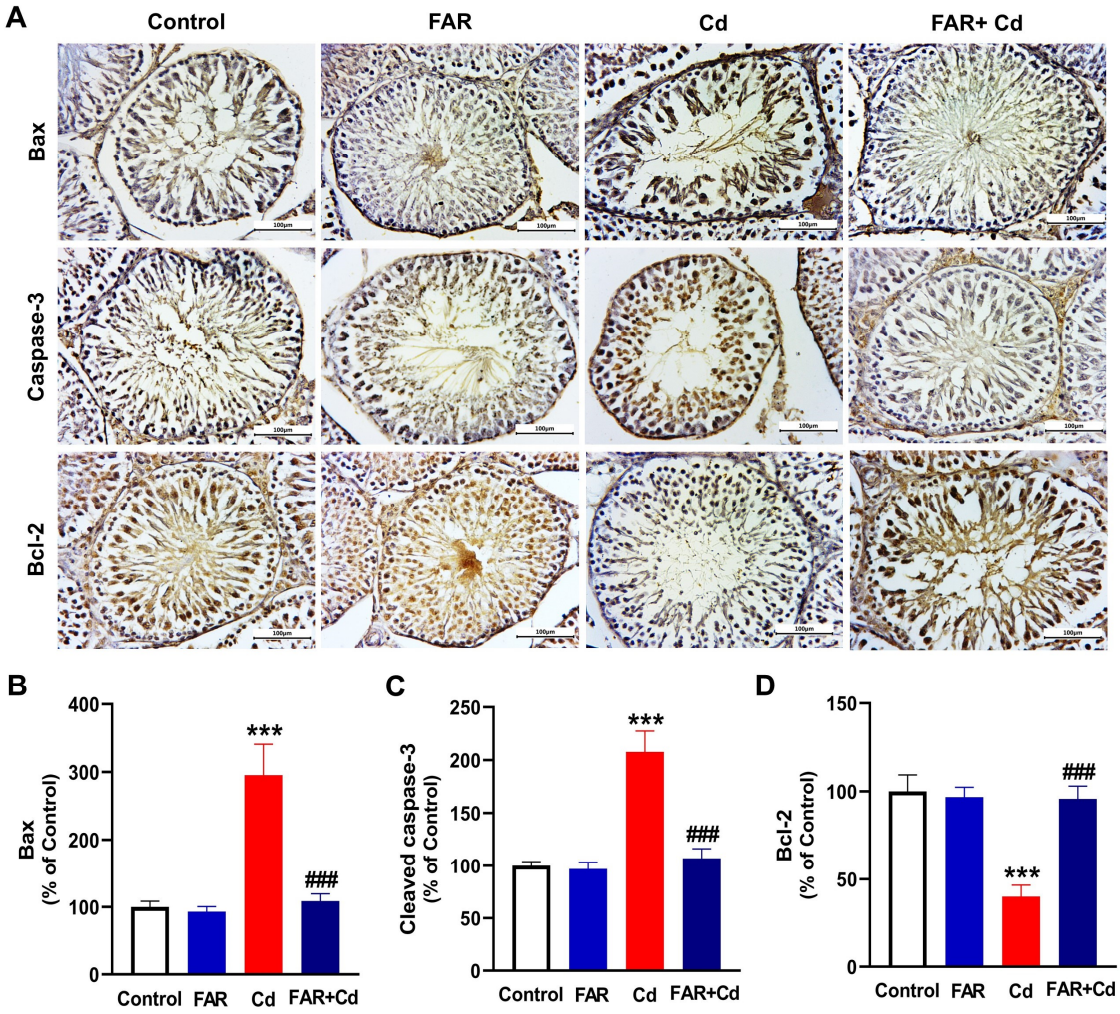

Figure 7. FAR prevented Cd-induced testicular apoptosis. FAR downregulated Bax (A, B) and cleaved caspase-3 (A, C), and increased Bcl-2 (A, D) expression levels in the testis of Cd-intoxicated rats. Data are mean ± SEM, (n = 6). ***P<0.001 versus Control. $^{###}$P<0.001 versus Cd.

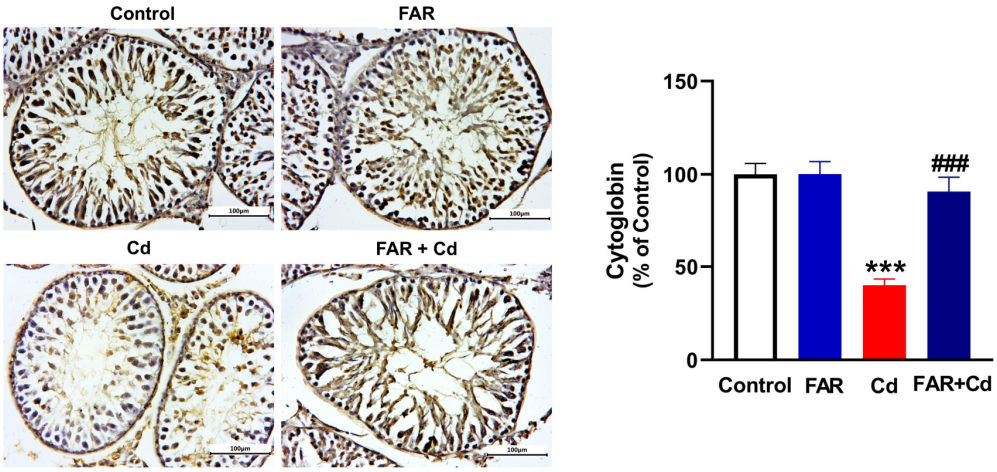

Figure 8. FAR upregulated cytoglobin in the testis of Cd-intoxicated rats. Data are mean ± SEM, (n = 6). ***P<0.001 versus Control. $^{###}$P<0.001 versus Cd.