Title

Diallyl disulfide prevents cadmium-induced testicular injury by attenuating oxidative stress, apoptosis, and TLR4/NF-κB and JAK1/STAT3 signaling and upregulating SIRT1 in rats

Authors and affiliation:

Emad H.M. Hassanein[1], Mohammed F. Alotaibi[2], Reem S. Alruhaimi[3], Omnia A.M. Abd El-Ghafar[4], Mostafa K. Mohammad[5], Ahmed M. Atwa[6] , Ayman M. Mahmoud[7]*

[1]Department of Pharmacology & Toxicology, Faculty of Pharmacy, Al-Azhar University-Assiut Branch, Egypt.

[2]Physiology Department, College of Medicine, King Saud University, Riyadh, 11461, Saudi Arabia.

[3]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia.

[4]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Nahda University, Beni-Suef, Egypt.

[5]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Badr University in Assiut, New Nasser City, West of Assiut, Egypt.

[6]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Egyptian Russian University, Cairo 11829, Egypt.

[7]Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester M1 5GD, UK.

*Corresponding author:

Ayman M. Mahmoud

Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester M1 5GD, UK

ORCID ID: 0000-0003-0279-6500

E-mail: a.mahmoud@mmu.ac.uk

Abstract

Background: Cadmium (Cd) is a heavy metal environmental pollutant that can cause serious health problems. Cd can cause structural changes in the testes and exposure to this heavy metal is associated with the loss of sperms and male infertility. The role of oxidative stress and inflammation in Cd toxicity has been acknowledged. Diallyl disulfide (DADS), an organo-sulfur compound found in garlic, possesses antioxidant, anti-inflammatory, and cytoprotective effects. This study evaluated the protective effect of DADS against Cd reproductive toxicity in male rats, emphasizing the involvement of redox imbalance, TLR-4/NF-κB and JAK1/STAT3 signaling, and SIRT1. **Methods**: DADS (10 mg/kg body weight) was administered orally to rats for 14 days and a single dose of Cd (1.2 mg/kg) was injected intraperitoneally on day 7. Blood and samples from the testes were collected for analysis. **Results**: Cd caused testicular injury manifested by multiple histopathological changes and loss of sperms from seminiferous tubules. Circulating levels of gonadotropins and testosterone were decreased in Cd-administered rats. DADS prevented Cd-induced testicular injury and ameliorated serum levels of gonadotropins and testosterone. Cd increased testicular reactive oxygen species (ROS) and malondialdehyde (MDA) and upregulated TLR-4, NF-κB, pro-inflammatory cytokines, JAK1 and STAT3 phosphorylation, Bax and caspase-3, while decreased antioxidants and Bcl-2. DADS effectively decreased ROS and MDA, downregulated TLR-4, NF-κB, JAK1, STAT3, pro-inflammatory cytokines and pro-apoptosis markers in Cd-administered rats. In addition, DADS enhanced antioxidants, Bcl-2, SIRT1 and cytoglobin in the testis of Cd-administered rats. **Conclusion**: DADS prevents Cd-induced testicular injury by attenuating oxidative stress, apoptosis, and TLR-4/NF-κB and JAK1/STAT3 signaling, and upregulating SIRT1 and antioxidants.

Keywords: Heavy metals; Garlic; Reproductive toxicity; Inflammation; Oxidative stress.

1. Introduction

Cadmium (Cd) is a heavy metal (HM) known for its toxic effect on the liver, kidney and reproductive organs [1-3]. Due to its long biological half-life, Cd accumulates in different tissues following occupational exposure and consumption of contaminated food and water [4-6]. According to several studies, the rapid cell division and metabolism of mammalian testes make them vulnerable to Cd toxicity [2]. Exposure to Cd can disrupt the blood-testis barrier, resulting in deleterious consequences on the testicles, sperm characteristics, and fertility [7, 8]. The toxic effects of Cd on the reproductive system and other organs are associated with oxidative stress (OS) provoked by excessive production of reactive oxygen species (ROS) [9-11]. Cd provokes ROS generation indirectly via Fenton reaction and other reactions provoked via free iron, and mitochondrial dysfunction [12, 13]. The generated superoxide ($\bullet O_2$) and hydroxyl ($\bullet OH$) radicals, and hydrogen peroxide (H_2O_2) attack cellular macromolecules resulting in protein malfunction, lipid peroxidation (LPO), DNA damage, and cell death [14]. OS is associated with inflammation due to the role of excess ROS in activating inflammatory pathways, such as toll-like receptor-4 (TLR-4)/nuclear factor-κB (NF-κB) signaling [15, 16]. Activation of TLR-4 and its downstream redox-sensitive transcription factor NF-κB promotes the release of pro-inflammatory cytokines which together with ROS alter mitochondrial function and elicit apoptotic cell death. These effects could have detrimental effects on fertility. In this context, testicular cell damage and declined testosterone synthesis and sexual behavior have been associated with Cd-induced activation of TLR-4/NF-κB signaling in piglets [17]. In addition, the Janus kinase (JAK)/signal transduction and transcriptional activator (STAT) signaling pathway regulates cellular responses to inflammation and increases organ damage [18]. Cytokines and other ligands can activate the JAK/STAT signaling which promotes cytokine-mediated cell activation. Upon binding to their

receptors, cytokines activate JAKs resulting in their phosphorylation and dimerization. Activated JAKs promote the phosphorylation of STATs followed by their dimerization and translocation into the nucleus to bind to DNA and inhibit or activate target genes [18, 19]. Despite the roles that JAK/STAT signaling plays in different biological processes, including cell differentiation, tissue repair and immune response, persistent activation of this signaling pathway can provoke inflammatory disorders. Previous findings demonstrated the role of ROS in activating JAK/STAT signaling and the association of its activation with OS and inflammatory responses [20, 21]. Accordingly, inhibitors of JAK have shown promising therapeutic effects in many clinical settings [22]. Therefore, mitigation of OS and suppression of TLR-4/NF-κB and JAK/STAT signaling pathways could be effective in protecting the testis against Cd toxicity.

Activation of the silent information regulator 2 homolog 1 (SIRT1) is associated with attenuation of OS, inflammation, and cell death [23, 24]. SIRT1 is a NAD^+-dependent histone deacetylase deeply involved in several cellular processes, including energy metabolism, genome stability, cell differentiation, and redox signaling [25, 26]. SIRT1 specifically protects against ROS and inflammation by regulating transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2), resulting in enhanced antioxidants and inhibition of NF-κB activation [25, 26]. Nrf2 controls the transcription of antioxidant and anti-inflammatory genes [27]. Given its key role in mitigating OS and inflammation, SIRT1 dysregulation is associated with several disorders [25, 26]. In this context, SIRT1-targeted anti-inflammatory therapies have shown promising clinical applications in treating inflammatory diseases [28]. Additionally, Nrf2 activation attenuated inflammation and oxidative damage induced by different agents such as HMs, hyperglycemia, pesticides, and chemotherapy [23, 29-31].

Plants represent an excellent source of several substances with beneficial pharmacological characteristics. One functional food that can help avoid many illnesses and toxicities is garlic [32]. The biological and health-promoting properties of garlic are thought to be mediated via its content of organic sulfur compounds, including diallyl disulfide (DADS) [33, 34]. DADS is made up of two allyl groups joined by two sulfur atoms, and showed anti-inflammatory, anti-cancer, and cytoprotective properties [33, 34]. In addition, DADS has demonstrated hepato-protective [35] and neuroprotective [36] effects, and protected the lung and the pancreas against inflammation and injury induced by cerulein [37]. The anti-inflammatory effects of DADS have been further demonstrated in microglia [38] and macrophages [39] challenged with lipopolysaccharide (LPS). However, nothing has yet been reported on the efficacy of DADS against Cd-induced testicular injury. This study investigated the protective potential of DADS against Cd-induced OS, inflammation and testicular injury, emphasizing the involvement of SIRT1 and TLR-4/NF-κB and JAK1/STAT3 signaling.

2. Materials and methods

2.1. Animals and treatments

Twenty-four male Wistar rats weighing 180–210 g were included in this investigation. The rats were housed under standard temperature (22 ± 1°C) and humidity (50-60%) on a 12 h dark-light cycle and given water and food *ad libitum*. The animal study protocol was approved by the ethics committee of Al-Azhar University (Assiut, Egypt) (AZ-AS\PH-REC\44\24). After a week of acclimatization, the animals were randomly allocated into four groups (n = 6) as follows:

Group I (Control): received 0.5% carboxymethyl cellulose (CMC) orally for 14 days.

Group II (DADS): received DADS (10 mg/kg) (Sigma, USA) in 0.5% CMC [40] orally for 14 days.

Group III (Cd): received 0.5% CMC orally for 14 days and 1.2 mg/kg $CdCl_2$ (Sigma, USA) dissolved in 0.9% saline [41] via intraperitoneal (i.p.) injection on day 7.

Group IV (DADS + Cd): received 10 mg/kg DADS orally for 14 days and a single i.p. injection of $CdCl_2$ (1.2 mg/kg) on days 7.

Groups I and II received a single i.p. injection of 0.9% saline on day 7. Twenty-four h after the last treatment, blood was collected via cardiac puncture under ketamine/xylazine anesthesia, and the animals were then sacrificed. The blood was centrifuged to separate serum and the animals were immediately dissected to remove the testes. Samples from the testes were fixed in 10% neutral buffered formalin (NBF), others were homogenized in cold Tris-HCl buffer (10 mM, pH = 7.4), centrifuged and the clear supernatant was collected and stored at -80°C. Other tissue samples were kept at -80°C.

2.2. Biochemical assays

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were assayed in the serum of rats using kits supplied by Elabscience (China). ROS was detected in the testicular tissue supernatant using 2′,7′-dichlorodihydrofluorescein diacetate as previously described [42]. Malondialdehyde (MDA) and reduced glutathione (GSH) levels, and superoxide dismutase (SOD) activity were determined in the testicular tissue supernatant using BioDiagnostics (Egypt) kits. Levels of testicular NF-κB p65, and the cytokines interleukin-1β (IL-1β) and tumor necrosis factor (TNF)-α were measured using (Cusabio, China) and Elabscience (China) ELISA kits, respectively. All assays were carried out following the manufacturers' instructions.

2.3. Histopathology and immunohistochemistry (IHC)

The tissue samples were fixed in 10% NBF for 24 h, dehydrated using an ascending series of ethanol, cleared in xylene, and embedded in paraffin. Four-µm sections were cut and stained with

hematoxylin and eosin (H&E) and Sirius red. Other sections were processed via IHC staining to determine changes in TLR-4, Bax, Bcl2, cleaved caspase-3, and cytoglobin. Briefly, the tissue sections were dewaxed, rehydrated in a descending series of ethanol, and then treated with 0.05 M citrate buffer (pH 6.8) and 0.3% H_2O_2. Following blocking, the sections were incubated overnight at 4°C with the primary antibodies (Biospes, China). After washing, the sections were incubated with secondary antibodies (Biospes, China). DAB in H_2O_2 was employed for color development and counterstaining was carried out using hematoxylin. ImageJ (NIH, USA) was used to measure the intensity of the developed color (6/rat).

2.4. Western blotting

To determine changes in JAK1 and STAT3 phosphorylation and SIRT1, frozen tissue samples were homogenized in RIPA buffer supplemented with proteinase/phosphatase inhibitors. The homogenate was centrifuged, the clear supernatant was collected, and protein content in the supernatant was measured using Bradford reagent. Forty µg protein was subjected to SDS-PAGE and the separated protein bands were transferred onto PVDF membranes. The membranes were blocked in 5% bovine serum albumin (BSA) and then probed with p-JAK1, JAK1, p-STAT3, STAT3, SIRT1, and β-actin primary antibodies (Santa Cruz Biotechnology, USA) overnight at 4°C. After washing, secondary antibodies were added for 1 h at room temperature and the membranes were washed. The protein bands were developed with the BCIP/NBT detection reagent and band intensity was measured using Image J (NIH, USA).

2.5. Statistical analysis

The findings are displayed as mean ± standard deviation (SD). Group comparisons were determined using one-way ANOVA followed by Tukey's test on GraphPad Prism 8. A P value <0.5 was considered significant.

3. Results

3.1. DADS prevents testicular injury and ameliorates pituitary-gonadal axis hormones in Cd-administered rats

H&E staining revealed normal seminiferous tubules and interstitial tissue with Leydig cells in control and DADS-treated rats (Fig. 1). In both groups, Sirius red staining showed the normal amount of collagen (Fig. 1). Cd administration resulted in decreased number of spermatogenic cells and spermatozoa, apoptotic changes, hemorrhage, edema, congested blood vessels, interstitial fibrosis, and aggregated inflammatory cells (Fig. 1). Treatment with DADS prevented Cd-induced tissue damage, interstitial fibrosis, and inflammatory cells infilteration, and increased the number of spermatogenic cells and spermatozoa (Fig. 1).

Data represented in Figure 2A-C revealed a significant decrease in circulating FSH (Fig. 2A), LH (Fig. 2B), and testosterone (Fig. 2C) in Cd-administered rats as compared to the control group ($P<0.001$). DADS increased serum levels of these hormones significantly in Cd-administered rats ($P<0.001$).

3.2. DADS attenuates testicular OS in Cd-administered rats

Cd administration increased ROS (Fig. 3A) and MDA levels (Fig. 3B) and decreased GSH content (Fig. 3C) and SOD activity (Fig. 3D) in rat testis ($P<0.001$). DADS significantly decreased testicular ROS and MDA and increased GSH and SOD in Cd-administered rats ($P<0.001$).

3.3. DADS suppresses TLR-4/NF-κB signaling in Cd-administered rats

IHC staining of TLR-4 revealed its significant upregulation in the testis of Cd-administered rats (Fig. 4A-B) as compared to the control rats ($P<0.001$). NF-κB p65 (Fig. 4C), TNF-α (Fig. 4D), and IL-1β (Fig. 4E) levels were significantly elevated in the testis of Cd-administered rats

(P<0.001). DADS downregulated testicular TLR-4, NF-κB p65, TNF-α, and IL-1β in Cd-administered rats (P<0.001).

3.4. DADS mitigates Cd-induced apoptosis in rat testis

The effects of Cd and/or DADS on Bax, caspase-3, and Bcl-2 expression are represented in Figure 5A-B. Cd administration upregulated testicular Bax and caspase-3 whereas decreased Bcl-2 expression significantly as compared to the control group (P<0.001). DADS downregulated Bax and caspase-3 and upregulated Bcl-2 in the testis of Cd-administered rats (P<0.001).

3.5. DADS downregulates JAK1/STAT3 signaling and upregulates SIRT1 and cytoglobin in Cd-treated rats

Cd increased JAK1 and STAT3 phosphorylation in the testis of rats (Fig. 6A-C) as compared to the control group (P<0.001). In contrast, SIRT1 was significantly decreased in the testis of Cd-administered rats (P<0.001; Fig. 6A,D). Similar to SIRT1, Cd downregulated cytoglobin in the testis of rats (P<0.001; Fig. 7). DADS markedly suppressed JAK1 and STAT3 phosphorylation and upregulated SIRT1 and cytoglobin in the testis of Cd-administered rats.

4. Discussion

Exposure to HMs, including Cd, is associated with male infertility as demonstrated in rodents and human epidemiological research [43]. Cd can cause severe injury to different structures of the testes, mainly seminiferous tubules, Sertoli cells (SCs), and blood-testis barrier. This damage leads to the loss of sperms and infertility [43]. OS and inflammation are key processes in the toxic mechanism of Cd [9-11], and a positive correlation between OS markers and urinary Cd levels in human has been reported [44]. This study showed the protective effect of DADS, an organosulfur compound with antioxidant and anti-inflammatory activities, against Cd-induced reproductive

toxicity in rats. Attenuation of OS, inflammation and apoptosis, and modulation of SIRT1 and TLR-4/NF-κB and JAK1/STAT3 signaling are involved in the protective mechanism of DADS.

Cd administration resulted in low serum levels of gonadotropins and testosterone associated with severe degenerative changes in rat testis. A decrease in the number of spermatogenic cells and spermatozoa, apoptotic changes, edema, interstitial fibrosis, congested blood vessels, hemorrhage, and aggregated inflammatory cells are histopathological changes observed in the testis of Cd-administered rats. These findings demonstrated significant reproductive toxicity in rats exposed to Cd. The declined levels of gonadotropins pinpointed the deleterious effect of Cd on the pituitary-gonadal axis, an effect that resulted in decreased testosterone secretion. In addition, the decline in testosterone is directly linked to apoptosis of Leydig cells. Studies have demonstrated the serious effect of Cd on seminiferous tubules, blood-testis barrier, and Leydig cells [43]. In the seminiferous tubules, SCs are essential for spermatogenesis through their role in the differentiation of spermatogonia and providing a link between seminiferous tubules and the interstitium [45]. Exposure of rodents to Cd provoked severe ultrastructure changes in SCs, including cytoplasmic vacuolation, mitochondrial damage, and altered cytoskeleton [46-48]. Defragmentation of actin filaments of SCs is another effect of Cd on the testis, ultimately resulting in disruption of the blood-testis barriers [48]. In accordance with our data, rats exposed to Cd exhibited disorganization of the seminiferous epithelium, decreased number of spermatogonia, and declined sperm motility, number, and viability [49-51]. DADS effectively protected the testis against the structural and functional alterations induced by Cd. These findings added support to studies demonstrated that DADS protected against testicular injury induced by cyclophosphamide [52] and irradiation [53]. Given the role of OS and inflammation in mediating Cd toxicity [12, 13, 49-51], the protective effect of DADS could be directly explained by its antioxidant and anti-inflammatory properties

[33, 34]. In this study, Cd administration provoked OS marled by elevated ROS and MDA and declined antioxidants. Additionally, Cd promoted an inflammatory response marked by upregulated TLR-4/NF-κB and pro-inflammatory cytokines. In this context, several studies have reported the role of OS in reproductive toxicity induced by Cd in both animal models [49-51] and human subjects [44]. *In vivo* studies using rodents revealed elevated LPO levels, DNA oxidative damage, and decreased antioxidant defenses [49-51]. Screening of OS markers and Cd levels in 1020 men revealed a positive correlation between urinary Cd and OS markers and a negative association with semen quality [44]. Cd can indirectly increase the production of ROS through Fenton reaction and mitochondrial dysfunction [12, 13]. Excess ROS provoke LPO and cause oxidative damage to proteins and DNA [14]. Elevated ROS and its consequent OS induced by Cd have been demonstrated not only in the testis but also in other organs such as liver, kidney and pancreas [54-57]. Cd causes a decline in antioxidants through binding to the sulfhydryl groups on GSH [58], and interacting with and disrupting the catalytic function of SOD and CAT [59, 60]. Besides OS, excess ROS promote an inflammatory response via activation of several signaling molecules, including TLR-4 and its downstream redox-sensitive NF-κB [15, 16]. Activation of TLR-4/NF-κB signaling leads to the release of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β. These cytokines work in concert with ROS to provoke cell death via mitochondrial damage and apoptosis [61]. Disruption of the mitochondrial membrane potential and subsequent release of cytochrome c activate caspase-3 which initiates the apoptotic cascade [62]. Accordingly, Cd induced apoptosis in the testis of rats as shown by the microscopic investigation and the upregulated Bax and caspase-3 along with downregulated Bcl-2. Moreover, Cd administration was associated with upregulated JAK1/STAT3 signaling evidenced by increased phosphorylation of JAK1 and STAT3 in rat testis. These data added additional support to the implication of OS and

inflammation in the mechanism underlying Cd reproductive toxicity. Excess ROS has been reported to be involved in JAK/STAT signaling activation [20, 21], a finding supported by the study of Khashab et al [63] who reported the involvement of JAK/STAT signaling in ROS-induced oxidative DNA damage in germ cells.

DADS effectively mitigated Cd-induced testicular OS, inflammation, and apoptosis, effects that were mediated, at least in part, via its antioxidant efficacy and suppression of TLR-4/NF-κB and JAK1/STAT3 signaling pathways. DADS decreased MDA, downregulated TLR-4, NF-κB, JAK1 and STAT3 phosphorylation, and pro-inflammatory cytokines, and enhanced antioxidants. Suppression of inflammation and OS was associated with significant protection against apoptosis as shown by Bax and caspase-3 suppression and Bcl-2 upregulation. These data demonstrated the the antioxidant and anti-inflammatory efficacies of DADS which have been reported in many *in vitro* and *in vivo* studies [64]. DADS enhanced antioxidant enzymes and prevented ROS generation in ethanol-challenged hepatocytes [65], LPS-treated macrophages [66], and H_2O_2-treated epithelial cells [67]. In mice challenged with carbon ion irradiation, DADS prevented mitochondrial dysfunction and apoptosis in the testis [53]. In a rat model of lead-induced reproductive toxicity, DADS suppressed LPO, downregulated caspase-3 and enhanced GSH and SOD in the testis [68]. In cyclophosphamide-administered rats, treatment with DADS decreased testicular MDA, prevented apoptosis and histopathological alterations, and improved the number of spermatogonia [69]. Other studies demonstrated the suppression of OS and inflammation as mechanisms of DADS protection against several disorders, including hepatotoxicity [70]. In murine colitis, DADS suppressed inflammation and submucosal edema [71]. It mitigated OS and acute inflammation in a murine paw edema model [72], and the transcriptional activity of NF-*κ*B in pancreatitis [73]. *In vitro* studies revealed the ability of DADS to suppress NF-*κ*B and prevent

inflammation in LPS-induced microglia [38] and macrophages [39]. Along with inflammation, DADS attenuated ROS production in deoxycholic acid-treated epithelial cells [74] and IL-1β-treated mesenchymal stem cells [75]. The current study introduced new information that DADS suppression of TLR-4/NF-*κ*B signaling is involved in the protective effect of DADS against Cd reproductive toxicity. Furthermore, this study pinpointed the suppressive effect of DADS on JAK1/STAT3 signaling in the testis of Cd-administered rats, adding more support to its anti-inflammatory efficacy. Activation of JAK/STAT signaling following Cd administration is directly linked to OS and the increase in pro-inflammatory cytokine. DADS effectively downregulated JAK1 and STAT3 phosphorylation, an effect that is attributed to attenuation of OS and inflammation.

To further explore the mechanism(s) underlying the protective effect of DADS on inflammation and OS provoked by Cd in rat testis, we demonstrated changes in SIRT1. This histone deacetylase plays a key role in redox signaling and inflammation [23, 24]. SIRT1 regulates different proteins involved in vital cellular processes such as Nrf2 and controls many antioxidant and anti-inflammatory genes [25-27]. The role of SIRT1 in spermatogenesis is mediated via its ability to influence the functions of SCs, Leydig cells, and spermatogonia [76]. The decline in SIRT1 provoked mitochondrial dysfunction, ROS generation, LPO, and oxidative DNA damage in sperms, resulting in infertility [77]. A strong negative correlation between seminal SIRT1 expression and the number, motility, and viability of sperms has been reported [78]. Given its role in mitigating OS and inflammation, upregulation of SIRT1 can protect the male reproductive system against Cd toxicity and disorders associated with oxidative damage. Interestingly, DADS upregulated testicular SIRT1 in Cd-administered rats, an effect that aligned with the suppressed OS, inflammation, and apoptosis. SIRT1 prevented H_2O_2-induced apoptosis in endothelial

progenitor cells via FOXO3a ubiquitination and degradation [79], and abolished caspase-mediated apoptosis in LPS-challenged PC12 cells [80]. The preventive effect of DADS on Cd-induced oxidative and inflammatory damage was further supported by cytoglobin upregulation. Cytoglobin possesses ROS-scavenging ability [81], and its suppression results in oxidative DNA damage and cell death [81]. Accordingly, cells and organs lacking cytoglobin are more vulnerable to radiation-induced fibrogenesis and inflammation [81]. In contrast, studies have demonstrated suppression of ROS generation and cell death via cytoglobin upregulation [82], as well as its suppressive effect on superoxide and peroxynitrite generation [83].

5. Conclusion

These findings introduce new information on the protective efficacy of DADS against Cd male reproductive toxicity. The protective mechanism of DADS included amelioration of the pituitary-gonadal axis hormones, and attenuation of histopathological alterations, OS, inflammation, and apoptosis. DADS suppressed LPO, TLR-4/NF-κB and JAK1/STAT3 signaling, and inflammatory mediators, and upregulated SIRT1 and antioxidants in the testis of Cd-administered rats. Therefore, DADS could be a valuable protective agent against Cd reproductive toxicity in individuals at risk. However, the lack of data showing the effect of DADS on sperm parameters could be considered a limitation of this study. Further studies to explore other mechanism(s) and clinical trials are recommended to determine the efficacy of DADS.

Declaration of competing interest

The authors declare no competing interests.

Data availability

The manuscript contains all data supporting the reported results.

Acknowledgment

Princess Nourah bint Abdulrahman University Researchers Supporting Project Number (PNURSP2024R381), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

References

[1] S. Satarug, S.H. Garrett, M.A. Sens, D.A. Sens, Cadmium, environmental exposure, and health outcomes, Ciencia & saude coletiva 16(5) (2011) 2587-2602.
[2] W. Ali, Y. Ma, J. Zhu, H. Zou, Z. Liu, Mechanisms of Cadmium-Induced Testicular Injury: A Risk to Male Fertility, Cells 11(22) (2022).
[3] Z.-G. Gong, X.-Y. Wang, J.-H. Wang, R.-F. Fan, L. Wang, Trehalose prevents cadmium-induced hepatotoxicity by blocking Nrf2 pathway, restoring autophagy and inhibiting apoptosis, Journal of inorganic biochemistry 192 (2019) 62-71.
[4] L. Järup, A. Akesson, Current status of cadmium as an environmental health problem, Toxicol Appl Pharmacol 238(3) (2009) 201-8.
[5] A. Åkesson, L. Barregard, I.A. Bergdahl, G.F. Nordberg, M. Nordberg, S. Skerfving, Non-renal effects and the risk assessment of environmental cadmium exposure, Environ Health Perspect 122(5) (2014) 431-8.
[6] T.S. Nawrot, J.A. Staessen, H.A. Roels, E. Munters, A. Cuypers, T. Richart, A. Ruttens, K. Smeets, H. Clijsters, J. Vangronsveld, Cadmium exposure in the population: from health risks to strategies of prevention, Biometals 23 (2010) 769-782.
[7] S. Kumar, A. Sharma, Cadmium toxicity: effects on human reproduction and fertility, Rev Environ Health 34(4) (2019) 327-338.
[8] L.L. Zhao, Y.F. Ru, M. Liu, J.N. Tang, J.F. Zheng, B. Wu, Y.H. Gu, H.J. Shi, Reproductive effects of cadmium on sperm function and early embryonic development in vitro, PLoS One 12(11) (2017) e0186727.
[9] S. Derouiche, T. Cheradid, M. Guessoum, Heavy metals, oxidative stress and inflammation in pathophysiology of chronic kidney disease-a review, Asian journal of pharmacy and technology 10(3) (2020) 202-206.
[10] Y. Li, H. Cui, D. Li, H.-Y. Fu, J.-Z. Li, W.-X. Xu, R.-F. Fan, Selenium alleviates pancreatic fibrosis in chickens caused by mercuric chloride: Involvement of the MAPK signaling pathway and selenoproteins, Environmental Pollution 357 (2024) 124448.
[11] Y. Li, H. Cui, W.X. Xu, H.Y. Fu, J.Z. Li, R.F. Fan, Selenium represses microRNA-202-5p/MICU1 aixs to attenuate mercuric chloride-induced kidney ferroptosis, Poult Sci 103(8) (2024) 103891.
[12] A. Cuypers, M. Plusquin, T. Remans, M. Jozefczak, E. Keunen, H. Gielen, K. Opdenakker, A.R. Nair, E. Munters, T.J. Artois, Cadmium stress: an oxidative challenge, Biometals 23 (2010) 927-940.
[13] L.-J. Yan, D.C. Allen, Cadmium-induced kidney injury: Oxidative damage as a unifying mechanism, Biomolecules 11(11) (2021) 1575.
[14] J.G. Paithankar, S. Saini, S. Dwivedi, A. Sharma, D.K. Chowdhuri, Heavy metal associated health hazards: An interplay of oxidative stress and signal transduction, Chemosphere 262 (2021) 128350.
[15] P.A. Baeuerle, V.R. Baichwal, NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules, Advances in immunology 65 (1997) 111-37.
[16] K. Asehnoune, D. Strassheim, S. Mitra, J.Y. Kim, E. Abraham, Involvement of reactive oxygen species in Toll-like receptor 4-dependent activation of NF-kappa B, Journal of immunology (Baltimore, Md. : 1950) 172(4) (2004) 2522-9.
[17] Y. Li, Y. Zhang, R. Feng, P. Zheng, H. Huang, S. Zhou, W. Ji, F. Huang, H. Liu, G. Zhang, Cadmium induces testosterone synthesis disorder by testicular cell damage via TLR4/MAPK/NF-κB signaling pathway leading to reduced sexual behavior in piglets, Ecotoxicol Environ Saf 233 (2022) 113345.

[18] P. Xin, X. Xu, C. Deng, S. Liu, Y. Wang, X. Zhou, H. Ma, D. Wei, S. Sun, The role of JAK/STAT signaling pathway and its inhibitors in diseases, Int Immunopharmacol 80 (2020) 106210.
[19] Z. Yan, S.A. Gibson, J.A. Buckley, H. Qin, E.N. Benveniste, Role of the JAK/STAT signaling pathway in regulation of innate immunity in neuroinflammatory diseases, Clin Immunol 189 (2018) 4-13.
[20] A.R. Simon, U. Rai, B.L. Fanburg, B.H. Cochran, Activation of the JAK-STAT pathway by reactive oxygen species, Am J Physiol 275(6) (1998) C1640-52.
[21] N.M. Al-Rasheed, N.M. Al-Rasheed, I.H. Hasan, M.A. Al-Amin, H.N. Al-Ajmi, A.M. Mahmoud, Sitagliptin attenuates cardiomyopathy by modulating the JAK/STAT signaling pathway in experimental diabetic rats, Drug Des Devel Ther 10 (2016) 2095-107.
[22] X. Hu, J. li, M. Fu, X. Zhao, W. Wang, The JAK/STAT signaling pathway: from bench to clinic, Signal Transduction and Targeted Therapy 6(1) (2021) 402.
[23] D.H. Sami, A.S. Soliman, A.A. Khowailed, E.H.M. Hassanein, E.M. Kamel, A.M. Mahmoud, 7-hydroxycoumarin modulates Nrf2/HO-1 and microRNA-34a/SIRT1 signaling and prevents cisplatin-induced oxidative stress, inflammation, and kidney injury in rats, Life Sci 310 (2022) 121104.
[24] A.M. Sayed, E.H.M. Hassanein, S.H. Salem, O.E. Hussein, A.M. Mahmoud, Flavonoids-mediated SIRT1 signaling activation in hepatic disorders, Life Sci 259 (2020) 118173.
[25] C. Chen, M. Zhou, Y. Ge, X. Wang, SIRT1 and aging related signaling pathways, Mech Ageing Dev 187 (2020) 111215.
[26] C.K. Singh, G. Chhabra, M.A. Ndiaye, L.M. Garcia-Peterson, N.J. Mack, N. Ahmad, The Role of Sirtuins in Antioxidant and Redox Signaling, Antioxid Redox Signal 28(8) (2018) 643-661.
[27] S. Satta, A.M. Mahmoud, F.L. Wilkinson, M. Yvonne Alexander, S.J. White, The Role of Nrf2 in Cardiovascular Function and Disease, Oxid Med Cell Longev 2017 (2017) 9237263.
[28] C. Granchi, F. Minutolo, Activators of Sirtuin-1 and their Involvement in Cardioprotection, Curr Med Chem 25(34) (2018) 4432-4456.
[29] R.S. Alruhaimi, A.F. Ahmeda, O.E. Hussein, M.F. Alotaibi, M.O. Germoush, H.A. Elgebaly, E.H.M. Hassanein, A.M. Mahmoud, Galangin attenuates chlorpyrifos-induced kidney injury by mitigating oxidative stress and inflammation and upregulating Nrf2 and farnesoid-X-receptor in rats, Environmental Toxicology and Pharmacology (2024) 104542.
[30] R.S. Alruhaimi, E.H.M. Hassanein, M.N. Bin-Jumah, A.M. Mahmoud, Cadmium-induced lung injury is associated with oxidative stress, apoptosis, and altered SIRT1 and Nrf2/HO-1 signaling; protective role of the melatonin agonist agomelatine, Naunyn Schmiedebergs Arch Pharmacol 397(4) (2024) 2335-2345.
[31] S.A. Antar, W. Abdo, R.S. Taha, A.E. Farage, L.E. El-Moselhy, M.E. Amer, A.S. Abdel Monsef, A.M. Abdel Hamid, E.M. Kamel, A.F. Ahmeda, A.M. Mahmoud, Telmisartan attenuates diabetic nephropathy by mitigating oxidative stress and inflammation, and upregulating Nrf2/HO-1 signaling in diabetic rats, Life Sci 291 (2022) 120260.
[32] M. Dorrigiv, A. Zareiyan, H. Hosseinzadeh, Garlic (Allium sativum) as an antidote or a protective agent against natural or chemical toxicities: A comprehensive update review, Phytother Res 34(8) (2020) 1770-1797.
[33] L.-Y. Sheen, C.-C. Wu, C.-K. Lii, S.-J. Tsai, Effect of diallyl sulfide and diallyl disulfide, the active principles of garlic, on the aflatoxin B1-induced DNA damage in primary rat hepatocytes, Toxicology Letters 122(1) (2001) 45-52.
[34] K. Gunasekaran, B.M.K. Vasamsetti, P. Thangavelu, K. Natesan, B. Mujyambere, V. Sundaram, R. Jayaraj, Y.-J. Kim, S. Samiappan, J.-W. Choi, Cytotoxic Effects of Nanoliposomal Cisplatin and Diallyl Disulfide on Breast Cancer and Lung Cancer Cell Lines, Biomedicines 11(4) (2023) 1021.
[35] I.C. Lee, S.H. Kim, H.S. Baek, C. Moon, S.H. Kim, Y.B. Kim, W.K. Yun, H.C. Kim, J.C. Kim, Protective effects of diallyl disulfide on carbon tetrachloride-induced hepatotoxicity through activation of Nrf2, Environ Toxicol 30(5) (2015) 538-48.

[36] G. Wang, Y. Yang, C. Wang, J. Huang, X. Wang, Y. Liu, H. Wang, Exploring the role and mechanisms of diallyl trisulfide and diallyl disulfide in chronic constriction-induced neuropathic pain in rats, Korean J Pain 33(3) (2020) 216-225.
[37] M.K. Marimuthu, A. Moorthy, T. Ramasamy, Diallyl disulfide attenuates STAT3 and NF-κB pathway through PPAR-γ activation in cerulein-induced acute pancreatitis and associated lung injury in mice, Inflammation 45(1) (2022) 45-58.
[38] H.Y. Park, N.D. Kim, G.Y. Kim, H.J. Hwang, B.W. Kim, W.J. Kim, Y.H. Choi, Inhibitory effects of diallyl disulfide on the production of inflammatory mediators and cytokines in lipopolysaccharide-activated BV2 microglia, Toxicol Appl Pharmacol 262(2) (2012) 177-84.
[39] C.C. Chu, W.S. Wu, J.P. Shieh, H.L. Chu, C.P. Lee, P.D. Duh, The Anti-Inflammatory and Vasodilating Effects of Three Selected Dietary Organic Sulfur Compounds from Allium Species, J Funct Biomater 8(1) (2017).
[40] Y. Hashizume, K. Shirato, I. Abe, A. Kobayashi, R. Mitsuhashi, C. Shiono, S. Sato, K. Tachiyashiki, K. Imaizumi, Diallyl disulfide reduced dose-dependently the number of lymphocyte subsets and monocytes in rats, J Nutr Sci Vitaminol (Tokyo) 58(4) (2012) 292-6.
[41] E.C. de Lima, C.F.G. de Moura, M.J.D. Silva, W. Vilegas, A.B. Santamarina, L.P. Pisani, F. de Oliveira, D.A. Ribeiro, Therapeutical properties of Mimosa caesalpiniifolia in rat liver intoxicated with cadmium, Environmental science and pollution research international 27(10) (2020) 10981-10989.
[42] W.G. Hozayen, A.M. Mahmoud, E.M. Desouky, E.S. El-Nahass, H.A. Soliman, A.A. Farghali, Cardiac and pulmonary toxicity of mesoporous silica nanoparticles is associated with excessive ROS production and redox imbalance in Wistar rats, Biomed Pharmacother 109 (2019) 2527-2538.
[43] Q. Zhu, X. Li, R.-S. Ge, Toxicological Effects of Cadmium on Mammalian Testis, Frontiers in Genetics 11 (2020).
[44] Y. He, L. Zou, W. Luo, Z. Yi, P. Yang, S. Yu, N. Liu, J. Ji, Y. Guo, P. Liu, X. He, Z. Lv, S. Huang, Heavy metal exposure, oxidative stress and semen quality: Exploring associations and mediation effects in reproductive-aged men, Chemosphere 244 (2020) 125498.
[45] D.M. Su, Y. Feng, L. Wang, Y.L. Wu, R.S. Ge, X. Ma, Influence of fetal Leydig cells on the development of adult Leydig cell population in rats, J Reprod Dev 64(3) (2018) 223-231.
[46] F. de Souza Predes, J.C. Monteiro, S.L. Matta, M.C. Garcia, H. Dolder, Testicular histomorphometry and ultrastructure of rats treated with cadmium and Ginkgo biloba, Biol Trace Elem Res 140(3) (2011) 330-41.
[47] P. Bizarro, S. Acevedo, G. Niño-Cabrera, P. Mussali-Galante, F. Pasos, M.R. Avila-Costa, T.I. Fortoul, Ultrastructural modifications in the mitochondrion of mouse Sertoli cells after inhalation of lead, cadmium or lead-cadmium mixture, Reprod Toxicol 17(5) (2003) 561-6.
[48] X. Xiao, D.D. Mruk, E.I. Tang, C.K.C. Wong, W.M. Lee, C.M. John, P.J. Turek, B. Silvestrini, C.Y. Cheng, Environmental toxicants perturb human Sertoli cell adhesive function via changes in F-actin organization mediated by actin regulatory proteins, Human Reproduction 29(6) (2014) 1279-1291.
[49] S. Jahan, A. Zahra, U. Irum, N. Iftikhar, H. Ullah, Protective effects of different antioxidants against cadmium induced oxidative damage in rat testis and prostate tissues, Syst Biol Reprod Med 60(4) (2014) 199-205.
[50] M.C. Cupertino, R.D. Novaes, E.C. Santos, A.C. Neves, E. Silva, J.A. Oliveira, S.L.P. Matta, Differential Susceptibility of Germ and Leydig Cells to Cadmium-Mediated Toxicity: Impact on Testis Structure, Adiponectin Levels, and Steroidogenesis, Oxid Med Cell Longev 2017 (2017) 3405089.
[51] V.U. Nna, G.A. Ujah, M. Mohamed, K.B. Etim, B.O. Igba, E.R. Augustine, E.E. Osim, Cadmium chloride-induced testicular toxicity in male wistar rats; prophylactic effect of quercetin, and assessment of testicular recovery following cadmium chloride withdrawal, Biomed Pharmacother 94 (2017) 109-123.
[52] S.-H. Kim, I.-C. Lee, H.-S. Baek, C. Moon, S.-H. Kim, J.-C. Kim, Protective effect of diallyl disulfide on cyclophosphamide-induced testicular toxicity in rats, Laboratory animal research 29(4) (2013) 204-211.

[53] C.-x. Di, L. Han, H. Zhang, S. Xu, A.-h. Mao, C. Sun, Y. Liu, J. Si, H.-y. Li, X. Zhou, Diallyl disulfide attenuated carbon ion irradiation-induced apoptosis in mouse testis through changing the ratio of Tap73/ΔNp73 via mitochondrial pathway, Scientific reports 5(1) (2015) 16020.
[54] R.S. Alruhaimi, E.H. Hassanein, M.N. Bin-Jumah, A.M. Mahmoud, Cadmium cardiotoxicity is associated with oxidative stress and upregulated TLR-4/NF-kB pathway in rats; protective role of agomelatine, Food and Chemical Toxicology 180 (2023) 114055.
[55] M. Umar Ijaz, M. Batool, A. Batool, K.A. Al-Ghanimd, S. Zafar, A. Ashraf, F. Al-Misned, Z. Ahmed, S. Shahzadi, A. Samad, U. Atique, N. Al-Mulhm, S. Mahboob, Protective effects of vitexin on cadmium-induced renal toxicity in rats, Saudi J Biol Sci 28(10) (2021) 5860-5864.
[56] E. Beytut, M. Aksakal, The effect of long-term supplemental dietary cadmium on lipid peroxidation and the antioxidant system in the liver and kidneys of rabbits, Turkish Journal of Veterinary & Animal Sciences 26(5) (2002) 1055-1060.
[57] J. Liu, W. Qu, M.B. Kadiiska, Role of oxidative stress in cadmium toxicity and carcinogenesis, Toxicology and applied pharmacology 238(3) (2009) 209-214.
[58] K. Renu, R. Chakraborty, H. Myakala, R. Koti, A.C. Famurewa, H. Madhyastha, B. Vellingiri, A. George, A. Valsala Gopalakrishnan, Molecular mechanism of heavy metals (Lead, Chromium, Arsenic, Mercury, Nickel and Cadmium) - induced hepatotoxicity – A review, Chemosphere 271 (2021) 129735.
[59] E. Casalino, G. Calzaretti, C. Sblano, C. Landriscina, Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium, Toxicology 179(1-2) (2002) 37-50.
[60] T. Wroñska-Nofer, J. Wisniewska-Knypl, E. Dziubaltowska, K. Wyszyñska, Prooxidative and genotoxic effect of transition metals (cadmium, nickel, chromium, and vanadium) in mice, Trace Elements and Electrocytes 16(2) (1999) 87-92.
[61] T. Lawrence, The nuclear factor NF-kappaB pathway in inflammation, Cold Spring Harb Perspect Biol 1(6) (2009) a001651.
[62] P. Chen, Y.-F. Hu, L. Wang, W.-F. Xiao, X.-Y. Bao, C. Pan, H.-S. Yi, X.-Y. Chen, M.-H. Pan, C. Lu, Mitochondrial apoptotic pathway is activated by H2O2-mediated oxidative stress in BmN-SWU1 cells from Bombyx mori ovary, PLoS One 10(7) (2015) e0134694.
[63] F. Khashab, F. Al-Saleh, N. Al-Kandari, F. Fadel, M. Al-Maghrebi, JAK Inhibition Prevents DNA Damage and Apoptosis in Testicular Ischemia-Reperfusion Injury via Modulation of the ATM/ATR/Chk Pathway, Int J Mol Sci 22(24) (2021).
[64] X. Song, Z. Yue, L. Nie, P. Zhao, K. Zhu, Q. Wang, Biological Functions of Diallyl Disulfide, a Garlic-Derived Natural Organic Sulfur Compound, Evid Based Complement Alternat Med 2021 (2021) 5103626.
[65] T. Zeng, C.L. Zhang, F.Y. Song, X.L. Zhao, L.H. Yu, Z.P. Zhu, K.Q. Xie, The activation of HO-1/Nrf-2 contributes to the protective effects of diallyl disulfide (DADS) against ethanol-induced oxidative stress, Biochim Biophys Acta 1830(10) (2013) 4848-59.
[66] I.S. Shin, J. Hong, C.M. Jeon, N.R. Shin, O.K. Kwon, H.S. Kim, J.C. Kim, S.R. Oh, K.S. Ahn, Diallyl-disulfide, an organosulfur compound of garlic, attenuates airway inflammation via activation of the Nrf-2/HO-1 pathway and NF-kappaB suppression, Food Chem Toxicol 62 (2013) 506-13.
[67] W. Jin, H. Wang, W. Yan, L. Xu, X. Wang, X. Zhao, X. Yang, G. Chen, Y. Ji, Disruption of Nrf2 enhances upregulation of nuclear factor-kappaB activity, proinflammatory cytokines, and intercellular adhesion molecule-1 in the brain after traumatic brain injury, Mediators Inflamm 2008 (2008) 725174.
[68] E. Hassan, K. Kahilo, T. Kamal, M. El-Neweshy, M. Hassan, Protective effect of diallyl sulfide against lead-mediated oxidative damage, apoptosis and down-regulation of CYP19 gene expression in rat testes, Life Sciences 226 (2019) 193-201.
[69] S.-H. Kim, I.-C. Lee, J.-W. Ko, I.-S. Shin, C. Moon, S.-H. Kim, J.-D. Heo, J.-C. Kim, Mechanism of protection by diallyl disulfide against cyclophosphamide-induced spermatotoxicity and oxidative stress in rats, Molecular & Cellular Toxicology 12(3) (2016) 301-312.

[70] S.X. Liu, H. Liu, S. Wang, C.L. Zhang, F.F. Guo, T. Zeng, Diallyl disulfide ameliorates ethanol-induced liver steatosis and inflammation by maintaining the fatty acid catabolism and regulating the gut-liver axis, Food Chem Toxicol 164 (2022) 113108.
[71] I. Fasolino, A.A. Izzo, T. Clavel, B. Romano, D. Haller, F. Borrelli, Orally administered allyl sulfides from garlic ameliorate murine colitis, Mol Nutr Food Res 59(3) (2015) 434-42.
[72] H. Zhang, C. Shang, Z. Tian, H.K. Amin, R.B. Kassab, A.E. Abdel Moneim, Y. Zhang, Diallyl Disulfide Suppresses Inflammatory and Oxidative Machineries following Carrageenan Injection-Induced Paw Edema in Mice, Mediators Inflamm 2020 (2020) 8508906.
[73] M. Mathan Kumar, R. Tamizhselvi, Protective effect of diallyl disulfide against cerulein-induced acute pancreatitis and associated lung injury in mice, Int Immunopharmacol 80 (2020) 106136.
[74] C. Feng, Y. Luo, Y. Nian, D. Liu, X. Yin, J. Wu, J. Di, R. Zhang, J. Zhang, Diallyl Disulfide Suppresses the Inflammation and Apoptosis Resistance Induced by DCA Through ROS and the NF-κB Signaling Pathway in Human Barrett's Epithelial Cells, Inflammation 40(3) (2017) 818-831.
[75] K. Bahrampour Juybari, T. Kamarul, M. Najafi, D. Jafari, A.M. Sharifi, Restoring the IL-1β/NF-κB-induced impaired chondrogenesis by diallyl disulfide in human adipose-derived mesenchymal stem cells via attenuation of reactive oxygen species and elevation of antioxidant enzymes, Cell Tissue Res 373(2) (2018) 407-419.
[76] C. Tatone, G. Di Emidio, A. Barbonetti, G. Carta, A.M. Luciano, S. Falone, F. Amicarelli, Sirtuins in gamete biology and reproductive physiology: emerging roles and therapeutic potential in female and male infertility, Hum Reprod Update 24(3) (2018) 267-289.
[77] F. Alam, H. Syed, S. Amjad, M. Baig, T.A. Khan, R. Rehman, Interplay between oxidative stress, SIRT1, reproductive and metabolic functions, Current Research in Physiology 4 (2021) 119-124.
[78] T. Mostafa, N. Nabil, L. Rashed, K. Makeen, M.A. El-Kasas, H.A. Mohamaed, Seminal SIRT1 expression in infertile oligoasthenoteratozoospermic men with varicocoele, Andrology 6(2) (2018) 301-305.
[79] Y.Q. Wang, Q. Cao, F. Wang, L.Y. Huang, T.T. Sang, F. Liu, S.Y. Chen, SIRT1 Protects Against Oxidative Stress-Induced Endothelial Progenitor Cells Apoptosis by Inhibiting FOXO3a via FOXO3a Ubiquitination and Degradation, J Cell Physiol 230(9) (2015) 2098-107.
[80] J. Ye, Z. Liu, J. Wei, L. Lu, Y. Huang, L. Luo, H. Xie, Protective effect of SIRT1 on toxicity of microglial-derived factors induced by LPS to PC12 cells via the p53-caspase-3-dependent apoptotic pathway, Neurosci Lett 553 (2013) 72-7.
[81] F.E. McRonald, J.M. Risk, N.J. Hodges, Protection from intracellular oxidative stress by cytoglobin in normal and cancerous oesophageal cells, PloS one 7(2) (2012) e30587.
[82] N.J. Hodges, N. Innocent, S. Dhanda, M. Graham, Cellular protection from oxidative DNA damage by over-expression of the novel globin cytoglobin in vitro, Mutagenesis 23(4) (2008) 293-8.
[83] J.L. Zweier, C. Hemann, T. Kundu, M.G. Ewees, S.A. Khaleel, A. Samouilov, G. Ilangovan, M.A. El-Mahdy, Cytoglobin has potent superoxide dismutase function, Proceedings of the National Academy of Sciences 118(52) (2021) e2105053118.

Figures:

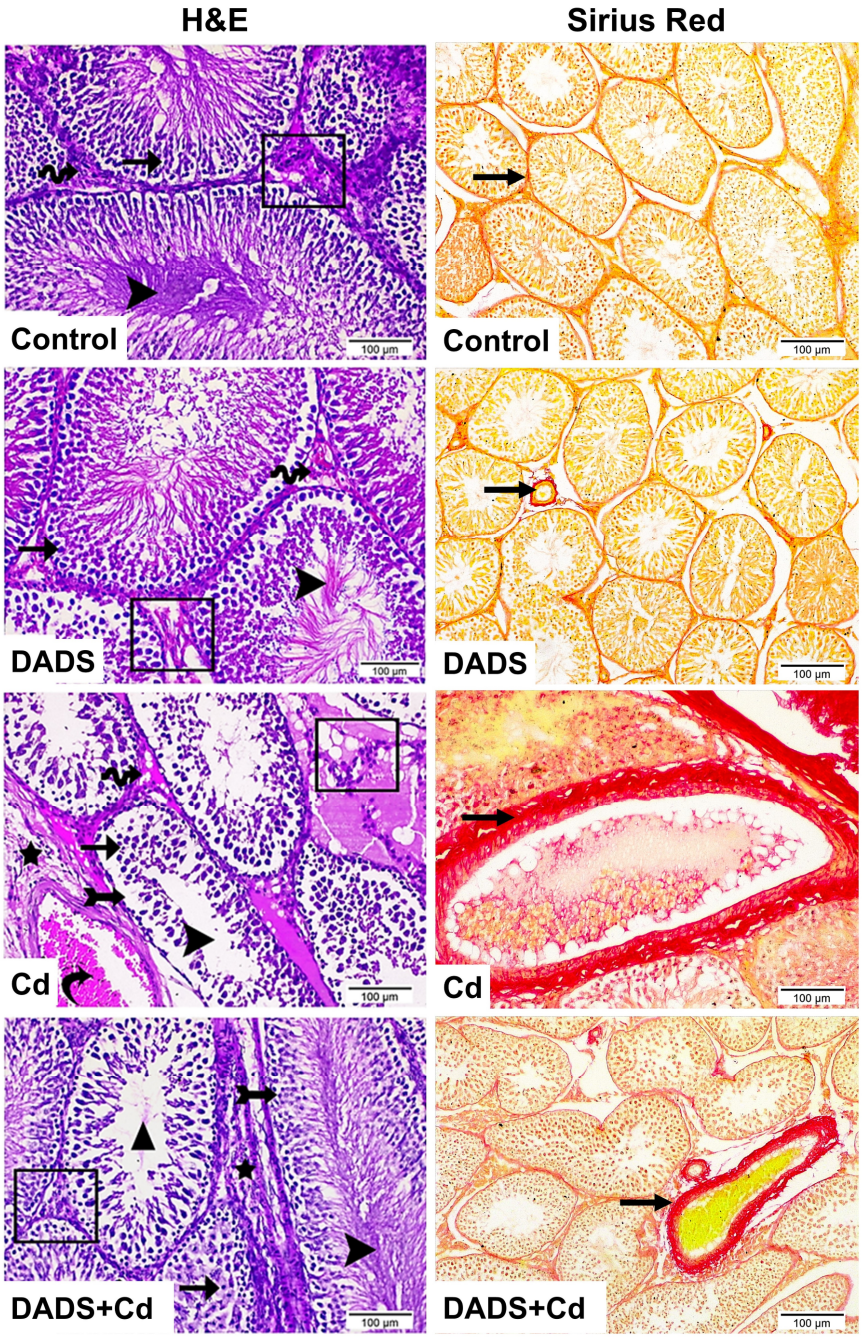

Figure 1. DADS prevented Cd-induced testicular injury. Photomicrographs of H&E-stained tsections in the testis of control and DADS-treated rats showing normal seminiferous tubules (arrow) with numerous sperms (arrowhead), interstitial tissue (rectangle), and Leydig cells (wave arrow); Cd-administered rats showing apoptotic changes in most of spermatogenic cells (arrow), edema (arrow with tail), tubular lumen appeared empty from spermatozoa (arrowhead), congested blood vessels (curved arrow), vacuolation (wave arrow), hemorrhage, deep basophilic

apoptotic Leydig cells (rectangle), and interstitial fibrosis along with aggregated inflammatory cells (star); and Cd-administered rats treated with DADS showing increase in spermatogenic cells (arrow with tail) except few tubules emerged with apoptotic spermatogenic cells (arrow), sperms (arrowhead), few tubules presented empty from any sperms (triangle), interstitial tissue (rectangle) presented with standard assembly of Leydig cells (wave arrow), and interstitial fibrosis as well as aggregated inflammatory cells (star) were still identified. Sirius red-stained sections in the testis of control and DADS-treated rats showing a regular amount of collagen (arrows); Cd-administered group showing increased collagen deposition and interstitial fibrosis (arrows); and Cd-administered rats treated with DADS showing decreased collagen deposition (arrow). (x200, Scale bar= 100 µm).

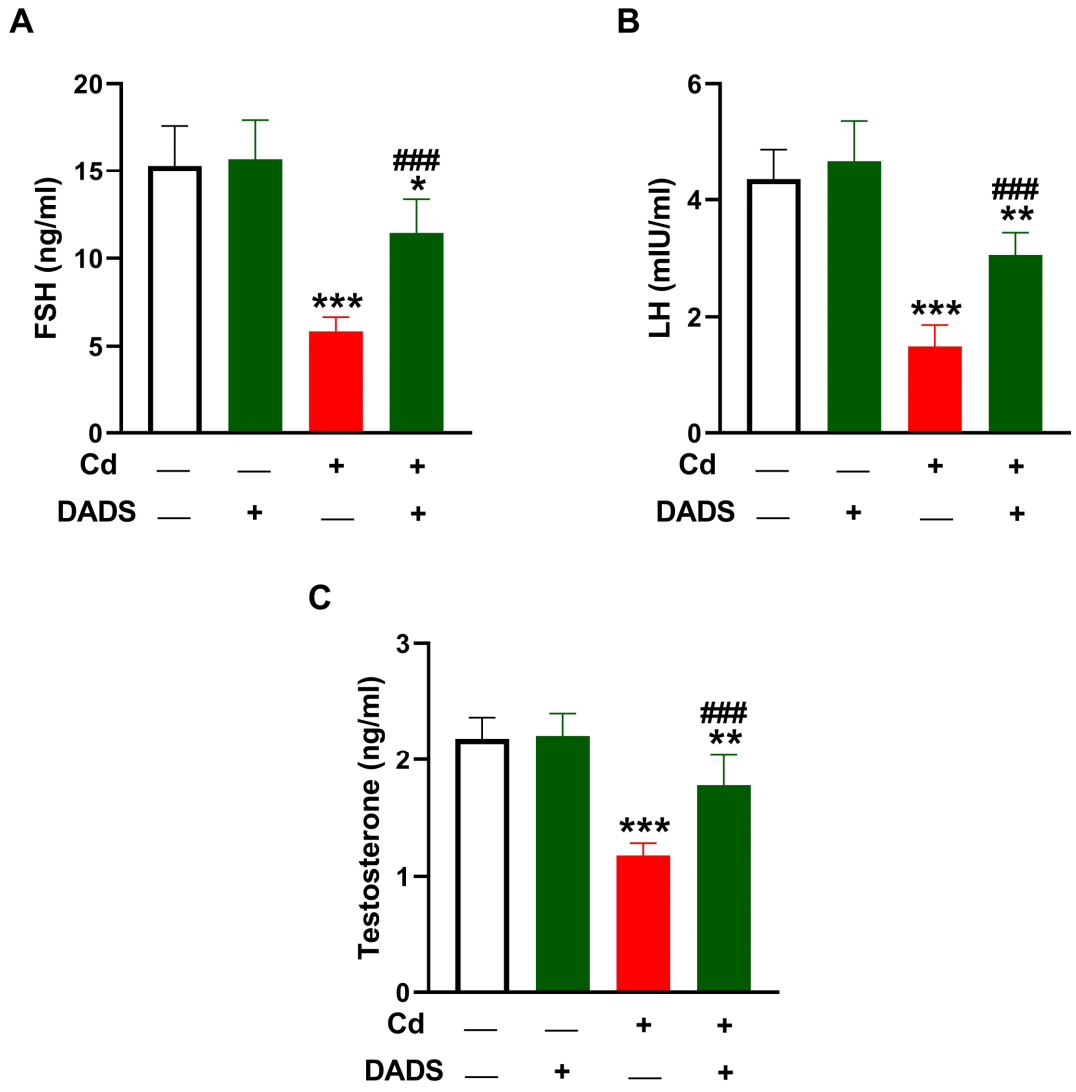

Figure 2. DADS increased serum FSH (A), LH (B), and testosterone (C) in Cd-administered rats. Data are mean ± SD, (*n* = 6). *P<0.05, **P<0.01, and ***P<0.001 versus Control. ###P<0.001 versus Cd.

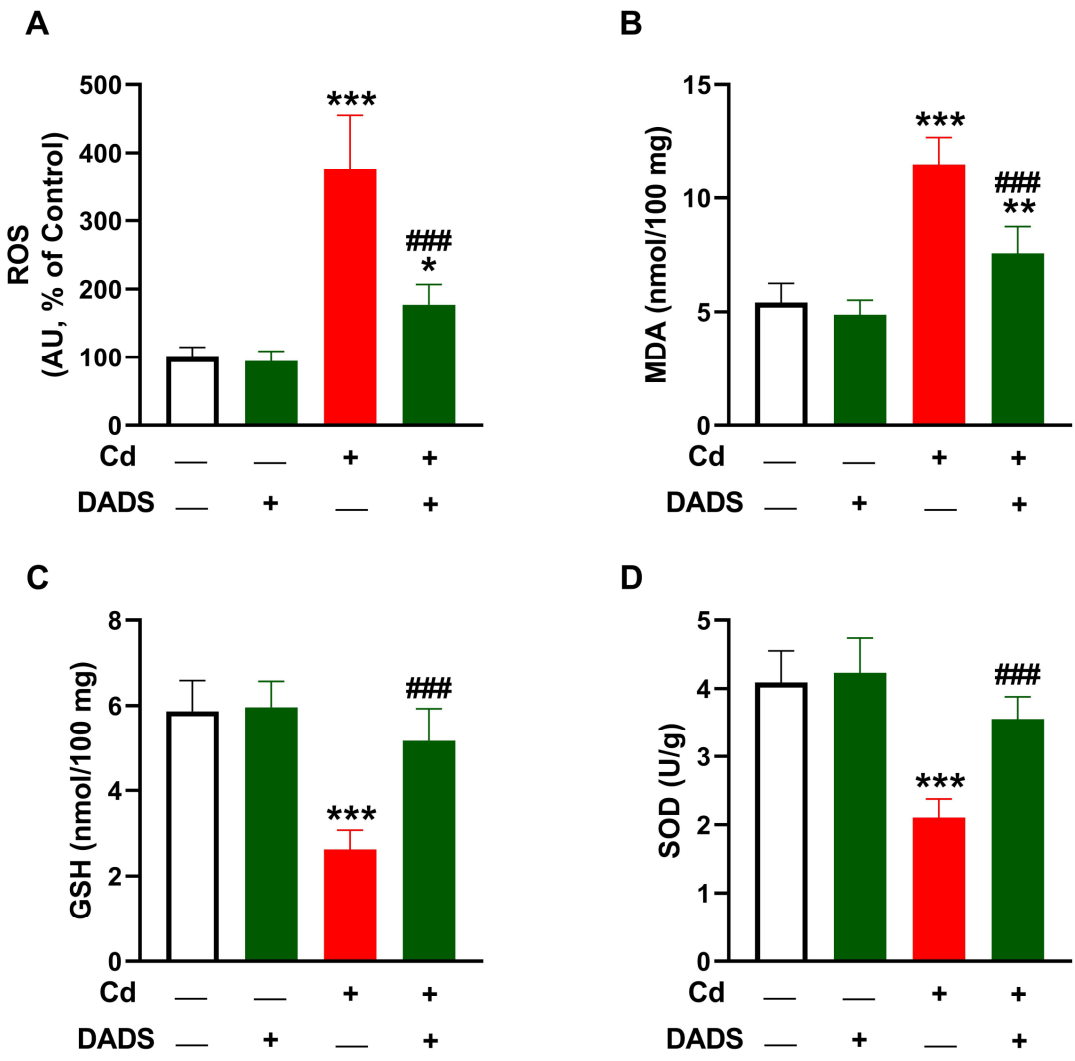

Figure 3. DADS decreased testicular ROS (A) and MDA (B), and increase GSH (C) and SOD (D) in Cd-administered rats. Data are mean ± SD, (*n* = 6). **P<0.01, and ***P<0.001 versus Control. ###P<0.001 versus Cd.

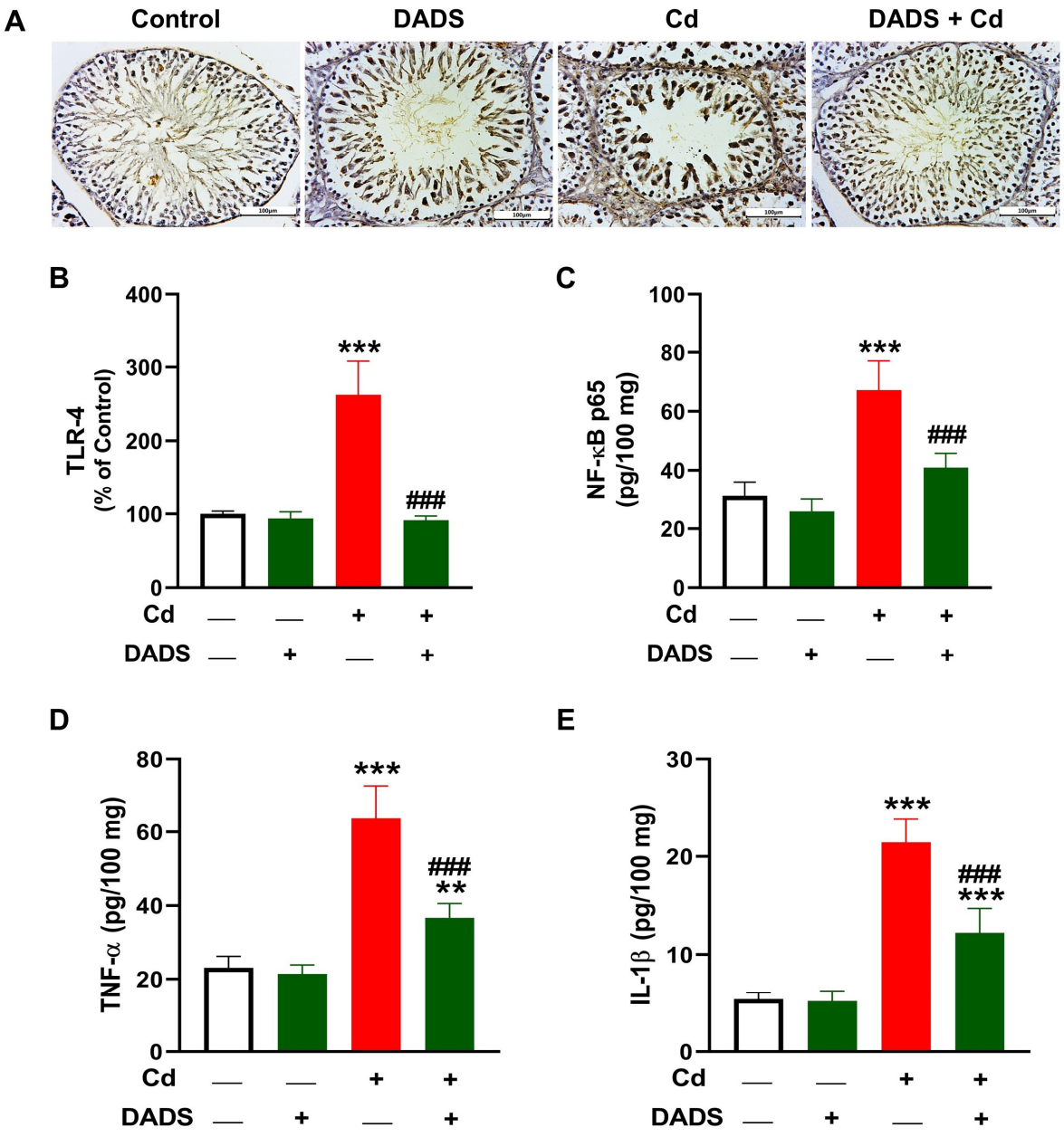

Figure 4. DADS downregulated TLR-4 (A,B), NF-κB p65 (C), TNF-α (D) and IL-1β (E) in the testis of Cd-administered rats. Data are mean ± SD, (n = 6). **P<0.01 and ***P<0.001 versus Control. ###P<0.001 versus Cd.

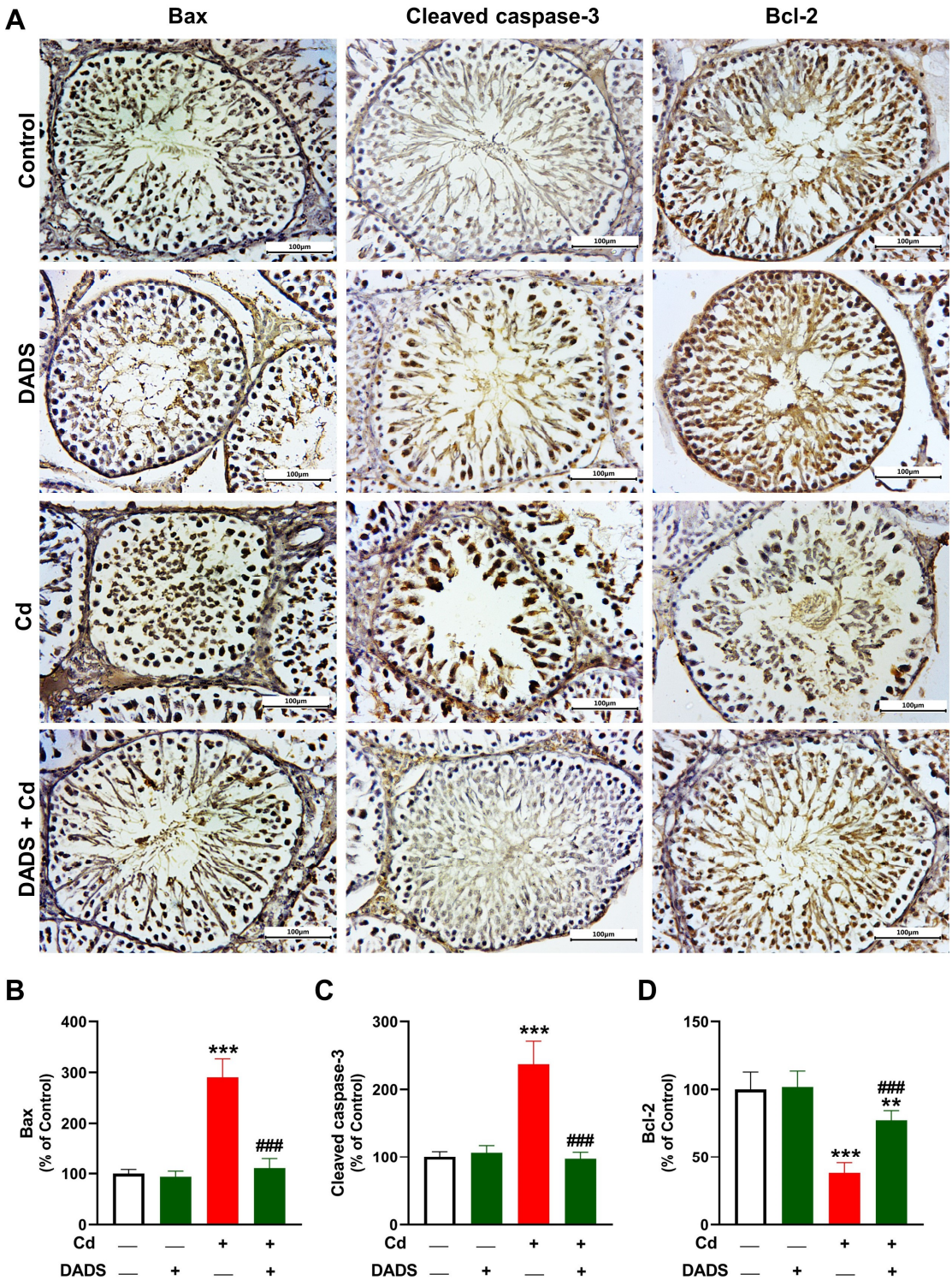

Figure 5. DADS attenuated apoptosis by downregulating Bax (A,B) and cleaved caspase-3 (A,C), and increasing Bcl-2 (A,D) in Cd-administered rats. Data are mean ± SD, (n = 6). **P<0.01 and ***P<0.001 versus Control. ###P<0.001 versus Cd.

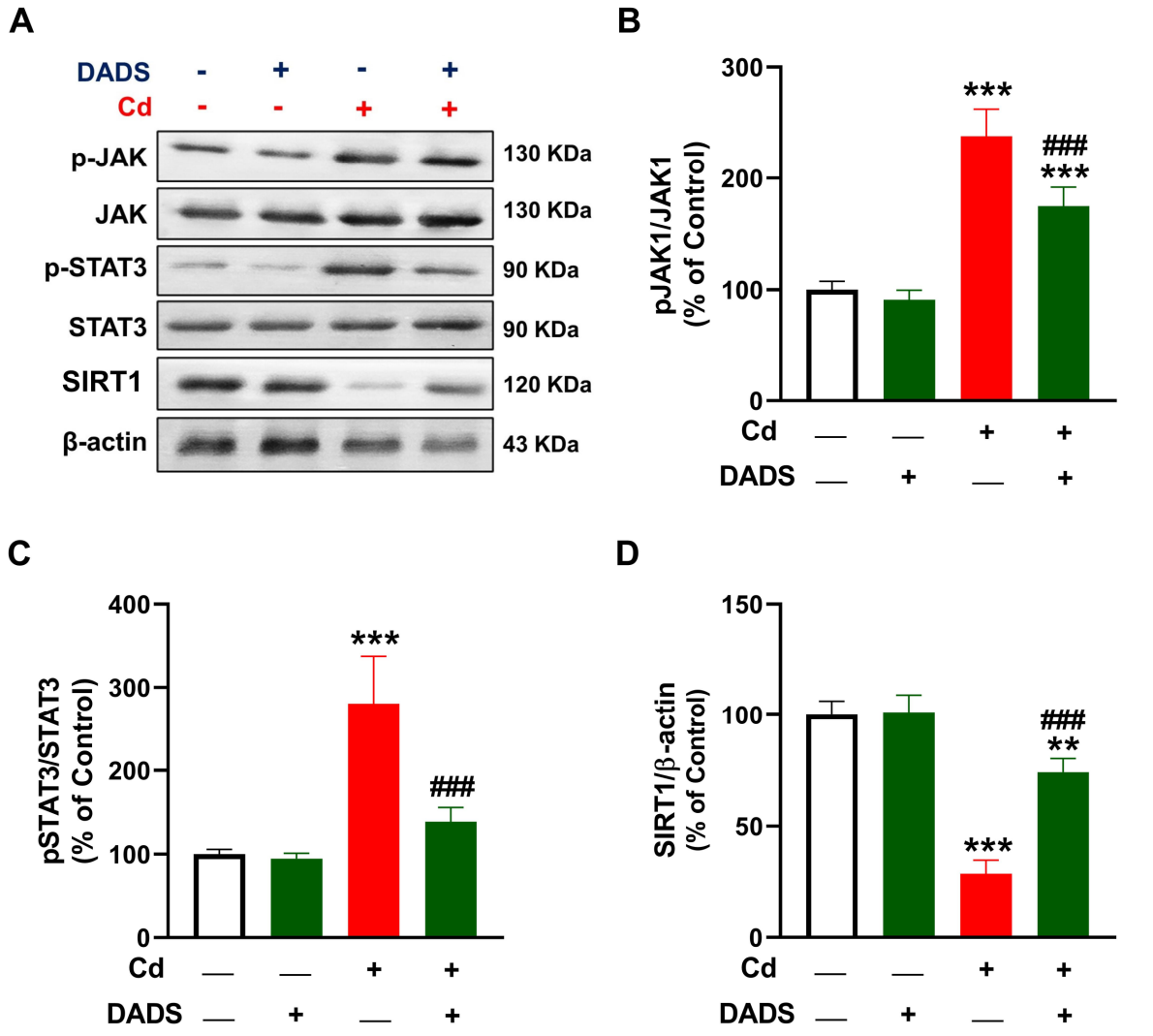

Figure 6. DADS suppressed JAK1/STAT3 signaling and upregulated SIRT1 in Cd-administered rats. DADS downregulated testicular JAK1 and STAT3 phosphorylation (A-C) and increased SIRT1 (A,D) in Cd-administered rats. Data are mean ± SD, (n = 6). **P<0.01 and ***P<0.001 versus Control. ###P<0.001 versus Cd.

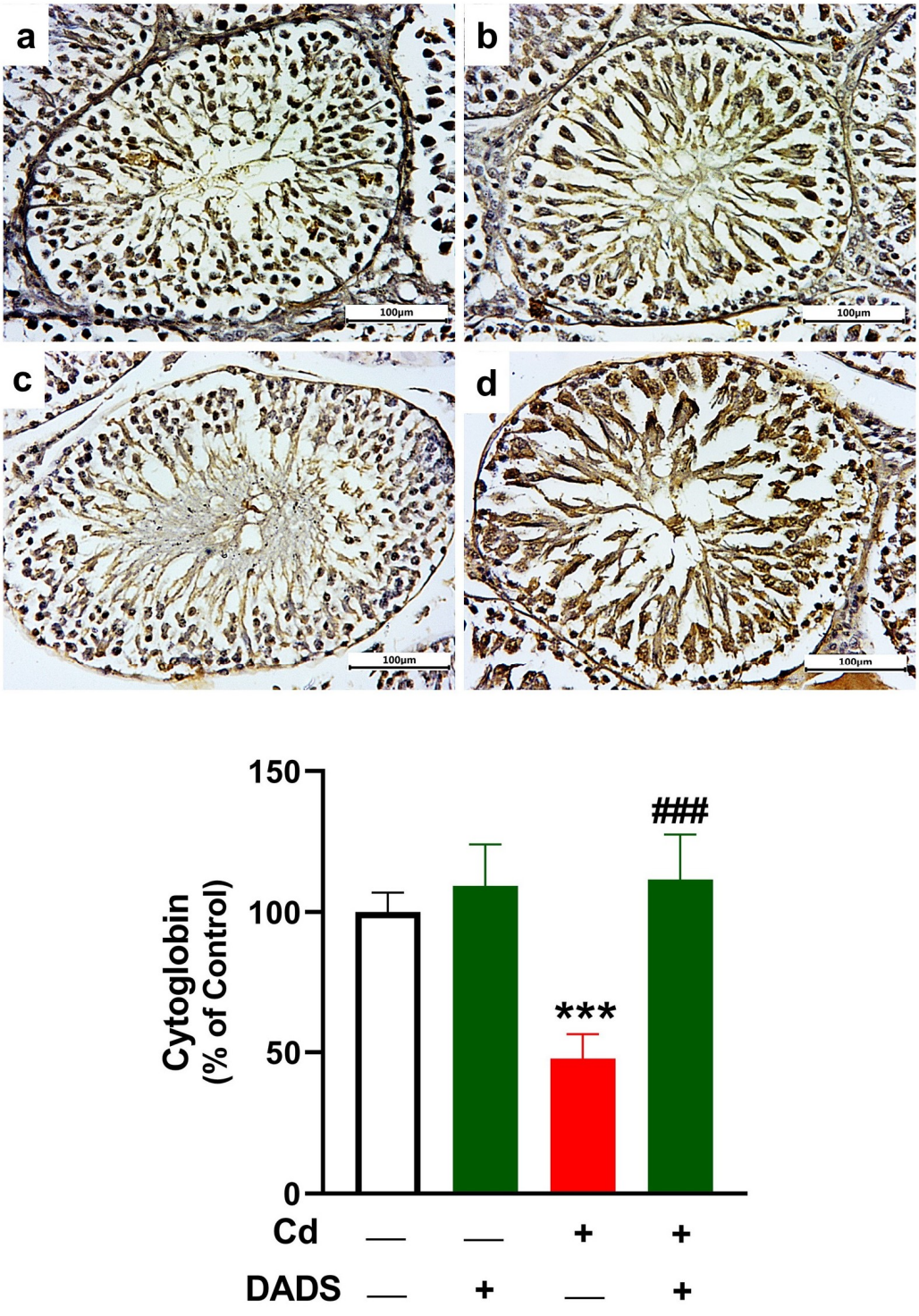

Figure 7. DADS increased cytoglobin in the testis of Cd-administered rats. Data are mean ± SD, (*n* = 6). ***P<0.001 versus Control. ###P<0.001 versus Cd.