

Mitigating cadmium-induced exposure in male rats: the protective role of *Ziziphus spina-christi*

Page 1 a 8

[Mitigando a exposição induzida por cádmio em ratos machos: O papel protetor de *Ziziphus spina-christi*]

A.A. Ammari¹ , A.R. Alhimaidei¹ , R.A. Amran¹ , Y. Al-anazi² , A.M. Rady¹ 

¹Department of Zoology, Faculty of Science, College of Science, King Saud University, Riyadh, Saudi Arabia

²Experimental animal care center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

ABSTRACT

Cadmium (Cd) is a pervasive environmental pollutant known for its detrimental effects on male reproductive health, causing testicular damage, hormonal imbalances, and reduced fertility. This study investigates the protective effects of *Ziziphus spina-christi* (Christ's Thorn Jujube) against cadmium-induced reproductive toxicity in male rats. Male rats were divided into four groups: a control group, a cadmium-only group (1mg/kg Cd), a *Ziziphus spina-christi* group (100mg/kg extract), and a cadmium plus *Ziziphus spina-christi* group. The treatments were administered for 21 days. Testis, epididymis, vas deferens, seminal vesicle, and prostate weights were measured. Hormone levels (LH, FSH, and testosterone). Histological examinations of testicular tissue were conducted to evaluate structural changes. Cadmium exposure significantly reduced the weights of all reproductive organs and decreased hormone levels, indicating severe reproductive toxicity. Co-treatment with *Ziziphus spina-christi* partially restored organ weights and improved hormonal levels. Histological analysis revealed significant pathological changes in the cadmium group, including disorganization of seminiferous tubules and reduction in spermatogenic cells. These changes were mitigated in the *Ziziphus spina-christi* treated groups, showing minimal structural damage. *Ziziphus spina-christi* demonstrated a protective effect against cadmium-induced reproductive toxicity, likely due to its antioxidant and anti-inflammatory properties.

Keywords: Cadmium, *Ziziphus spina-christi*, reproductive toxicity, male fertility, antioxidants, hormonal balance, histological analysis

RESUMO

O cádmio (Cd) é um poluente ambiental generalizado conhecido por seus efeitos prejudiciais à saúde reprodutiva masculina, causando danos aos testículos, desequilíbrios hormonais e redução da fertilidade. Este estudo investiga os efeitos protetores da *Ziziphus spina-christi* (Jujuba espinhosa de Cristo) contra a toxicidade reprodutiva induzida pelo cádmio em ratos machos. Os ratos machos foram divididos em quatro grupos: um grupo de controle, um grupo somente de cádmio (1mg/kg de Cd), um grupo de *Ziziphus spina-christi* (100mg/kg de extrato) e um grupo de cádmio mais *Ziziphus spina-christi*. Os tratamentos foram administrados por 21 dias. Foram medidos os pesos do testículo, epidídimo, canal deferente, vesícula seminal e próstata. Níveis hormonais (LH, FSH e testosterona). Foram realizados exames histológicos do tecido testicular para avaliar as alterações estruturais. A exposição ao cádmio reduziu significativamente os pesos de todos os órgãos reprodutivos e diminuiu os níveis de hormônio, indicando toxicidade reprodutiva grave. O co-tratamento com *Ziziphus spina-christi* restaurou parcialmente os pesos dos órgãos e melhorou os níveis hormonais. A análise histológica revelou alterações patológicas significativas no grupo do cádmio, incluindo a desorganização dos túbulos seminíferos e a redução das células espermatogênicas. Essas alterações foram atenuadas nos grupos tratados com *Ziziphus spina-christi*, que apresentaram danos estruturais mínimos. O *Ziziphus spina-christi* demonstrou um efeito protetor contra a toxicidade reprodutiva induzida pelo cádmio, provavelmente devido às suas propriedades antioxidantes e anti-inflamatórias.

Palavras-chave: Cádmio, *Ziziphus spina-christi*, toxicidade reprodutiva, fertilidade masculina, antioxidantes, equilíbrio hormonal, análise histológica

INTRODUCTION

Cadmium (Cd), a contaminant of emerging concern due to bio-accumulative properties and significant toxicity. At low exposure levels Cd affects kidneys, bones, and reproductive health. Its widespread release from industrial sources and presence in agriculture contribute to human and ecological risks. Due to challenges in removal and ongoing health impacts, cadmium is recognized as a contaminant of emerging concern globally. Exposure to cadmium occurs via many sources, including industrial emissions, contaminated food and water, and tobacco smoke. One of the critical health concerns associated with cadmium exposure is its detrimental impact on male reproductive health. Cadmium has been reported to induce testicular damage, reduce sperm quality, and disrupt hormonal balance, leading to impaired fertility in males (Hocaoglu-Özyiğit *et al.*, 2020). The mechanisms through which cadmium exerts its toxic effects on the male reproductive system are multifaceted. Cadmium can induce oxidative stress, which leads to lipid peroxidation, DNA damage, and apoptosis in testicular cells. Additionally, cadmium disrupts the blood-testis barrier, impairing spermatogenesis and resulting in decreased sperm count and motility (Anyanwu *et al.*, 2020). Chronic exposure to cadmium has been associated with decreased levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), further contributing to its negative impact on male fertility.

Recently, there is a growing effort in bioprospecting research to identify natural compounds that can mitigate the adverse effects of cadmium on male fertility. In this regard, plants have always been the reservoir for identifying small molecules of medicinal purpose including those that mitigate Cd reproductive toxicity. More importantly, *Ziziphus spina-christi*, commonly known as Christ's Thorn Jujube, is a plant with a long history of medicinal use in various cultures. Extracts from *Ziziphus spina-christi* have been reported to possess antioxidant, anti-inflammatory, and antimicrobial properties (Mahmoud *et al.*, 2022), making it a promising candidate for protecting against cadmium-induced reproductive toxicity. Studies have shown that the bioactive compounds in *Ziziphus spina-christi*, such as flavonoids,

saponins, and polyphenols, can scavenge free radicals and enhance the antioxidant defense system in the body (Dhanalekshmi *et al.*, 2022). These properties suggest that *Ziziphus spina-christi* exert counteracting activity against the oxidative stress and cellular damage caused by cadmium exposure. Additionally, the anti-inflammatory effects of *Ziziphus spina-christi* may contribute to its protective role by reducing inflammatory-induced damage in the testicular tissue. This study aims to investigate the protective effects of *Ziziphus spina-christi* against cadmium-induced infertility in male rats. By examining the impact of *Ziziphus spina-christi* on the quality and quantity of rat sperm, testicular histopathology, and hormonal levels in cadmium-exposed rats, we aim to elucidate the potential mechanisms through which this plant may confer its protective effects. Understanding these mechanisms may provide valuable insights into developing natural therapeutic strategies for mitigating heavy metal-induced reproductive toxicity.

MATERIALS AND METHODS

The leaves of the plant of interest (*Ziziphus spina-christi*) were obtained in Damman area (26.43°N 50.1°E) from October to December 2023. The leave samples were harvested directly from the plant, cleaned, and air dried in dark room. Once dried, the leaves were ground into a powder. The powdered *Ziziphus spina-christi* leaves were macerated in water for 24 hours. The solution was then filtered using Whatman paper. After maceration, the *Ziziphus spina-christi* extract was evaporated at a temperature range of 50 to 60°C. The concentrate was prepared as a stock solution at 100kg/mL concentration, then frozen at -20 °C (Ammari *et al.*, 2024).

Viable male rats were acquired from the animal facility at the Zoology Department, Science College, King Saud University (KSU). The rats were acclimated to a well-ventilated environment with a stable room temperature of 25±2 °C, a regular 12-hour light/dark cycle, and were provided with a standard diet and water ad libitum. All experimental procedures followed the guidelines specified by the ethics committee (Approval no: KSU-SE-24-3) and the Institutional Animal Care at KSU. The study involved twelve male rats, each weighing between 200-240 grams and aged between 12-15

weeks. The rats were divided into four groups, each containing three rats: the control group (G-control) received no dosage; Cd-1mg/kg group (second group) were exposed to 1mg/kg cadmium; Zsc-100mg/kg group (third group) were administered with 100mg/mL *Ziziphus spina-christi* extract; and Cd + Zsc group (fourth group) received both cadmium and *Ziziphus spina-christi* extract for 21 days.

After the completion of the treatments on 21st day, blood samples were collected from the hearts of the animals into non-heparinized tubes and maintained at 4°C overnight. The clotted blood was centrifuged at 1000 xg for 15 minutes to obtain serum. After centrifugation, the serum obtained were kept at -20 °C for hormonal studies. An array of biochemical measurements was conducted on serum samples to investigate various mechanistic aspects. These included the assessment of biological markers and oxidative stress. Specifically, fertility markers were evaluated using enzyme-linked immunosorbent assay (ELISA) kits designed for rats including Follicle-stimulating hormone (FSH) (Catalog No. EK720702) Luteinizing hormone (LH) (Catalog No. MBS2018978) Testosterone (Catalog No. EK720991). All obtained from ̳AFG Bioscience, Skokie Boulevard Northbrook, IL 60062 USA.

The testes of the experimental animals were surgically excised after the animals were anesthetized with CO₂. The excised testes were immediately fixed in 10% neutral formalin for 48 hours. Thereafter, histological sections were made from the fixed testes. Basically, samples were dehydrated using a series of alcohol solutions, infiltrated with xylene for 4-6 hours, embedded in paraffin wax cubes, sectioned using a microtome, mounted on slides, and stained with hematoxylin and eosin (H&E). Histomorphology and histomorphometry evaluations were conducted using a light microscope.

All statistical analyses from this experiment were performed using the GraphPad Prism (10.1.1) software. The Shapiro-Wilk test confirmed the normal distribution of the data. Body weights of the animals were analyzed using two-way ANOVA (considering day factor and extract factor) and Tukey's multiple comparisons test. Hormone levels, reproductive organ weights, were analyzed using one-way ANOVA, followed

by Tukey's test. Results are presented as mean values with standard deviations, and a P value of 0.05 or less was considered statistically significant.

RESULTS

The examination of the effect of Cd exposure on the reproductive health in male rat was histologically studied. Comparing the tissue architecture of the control group with the treated and exposed groups, the control group demonstrates normal architecture of seminiferous tubules with well-organized spermatogenic cells. Fig1 shows that interstitial space in control is minimal and contains Leydig cells. Spermatogonia, primary and secondary spermatocytes, and spermatids are clearly distinguishable and the Sertoli cells support the structure. However, in the Cadmium exposed group, significant pathological changes including disorganization of seminiferous tubules, reduction in spermatogenic cells, and vascular congestion was evidenced post 21-day exposure. Meanwhile, the administration of *Ziziphus spina-christi* in the third group demonstrates no pathological changes, indicating a protective or neutral effect on testicular tissue. Interestingly, the treatment of Cadmium exposed group with 100mg/kg with *Ziziphus spina christi* demonstrates a mild pathological change, indicating a mitigating effect of *Ziziphus spina-christi* on cadmium-induced testicular damage.

The effect of Cd exposure and mitigating effects of *Ziziphus spina-christi* on the weight of male rat reproductive organs including testis, epididymis, vas deferens, seminal vesicle, and prostate were recorded. The study assessed the impact of cadmium exposure on the weights of reproductive organs in a controlled experiment. The results indicated that cadmium significantly reduced the weights of the testis (from 1.7g in the control group to 0.7g), epididymis (0.9g to 0.4g), vas deferens (0.5g to 0.3g), seminal vesicles (1.2g to 0.6g), and prostate (0.5g to 0.3g). In contrast, administration of *Ziziphus spina-christi* in rats improved the reproductive organ weights, with the testis weight increasing to 1.8 g, the epididymis to 1.0g, and the seminal vesicles to 1.3g. *Ziziphus spina-christi* showed potential as a natural remedy to counteract the adverse effects of cadmium Fig. 1.

Furthermore, the co-treatment of cadmium with *Ziziphus spina-christi* also demonstrated beneficial effects, resulting in partial recovery of organ weights compared to the cadmium group. Testis weight increased to approximately 1.3g, epididymis weight to 0.6g, vas deferens weight to 0.4g, seminal vesicle weight to 0.8g, and prostate weight to 0.4g. These findings suggest that *Ziziphus spina-christi* may offer a protective effect against cadmium-induced toxicity in reproductive organs, highlighting its potential role in mitigating the harmful consequences of heavy metal exposure Fig. 2.

The male reproductive system is closely controlled by a complex interplay of hormones, including testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), each of which contributes to various physiological processes. The result from this study shows the effects of cadmium exposure and treatment with *Ziziphus spina-christi* on hormone levels in male rats, specifically focusing on Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and Testosterone. The results show that cadmium exposure significantly impacted hormone production, with notable alterations in LH levels. In the control group, average LH levels were approximately 16 pg/ml, while cadmium exposure reduced these levels to around 14pg/mL. Conversely, the administration of *Ziziphus spina-christi* alone led to an increase in LH levels to about 18 pg/mL, suggesting its potential to enhance LH production. Moreover, the treatment of cadmium exposed group with *Ziziphus spina-christi* treatment shows that the level of LH was

maintained at approximately 18 pg/ml, indicating a protective effect against cadmium-induced changes Fig. 3.

Similar trends were observed in the assessment of Follicle Stimulating Hormone (FSH). In the control group, FSH levels averaged around 8pg/mL, but cadmium exposure resulted in a significant reduction to approximately 4pg/mL. However, treatment with *Ziziphus spina-christi* alone improved FSH levels to about 10pg/mL. While the co-treatment of cadmium with *Ziziphus spina-christi* led to an increase in FSH levels to around 6 pg/ml, it did not fully restore the levels to those observed in the control group. This indicates a partial protective effect of *Ziziphus spina-christi* against the suppression of FSH due to cadmium exposure, highlighting its role in supporting hormonal balance in the presence of toxins Fig. 3.

Testosterone levels were also significantly affected by cadmium exposure, dropping from approximately 16pg/mL in the control group to around 8 pg/ml in the cadmium group. Treatment with *Ziziphus spina-christi* resulted in an increase in testosterone levels to about 18pg/mL, surpassing the control levels. In the group receiving both cadmium and *Ziziphus spina-christi* treatment, testosterone levels rose to approximately 12pg/mL, indicating a degree of protection against the testosterone-reducing effects of cadmium. Overall, the results suggest that *Ziziphus spina-christi* may enhance hormonal function and offer protective benefits against the detrimental effects of cadmium on reproductive hormone levels in male rats Fig. 3.

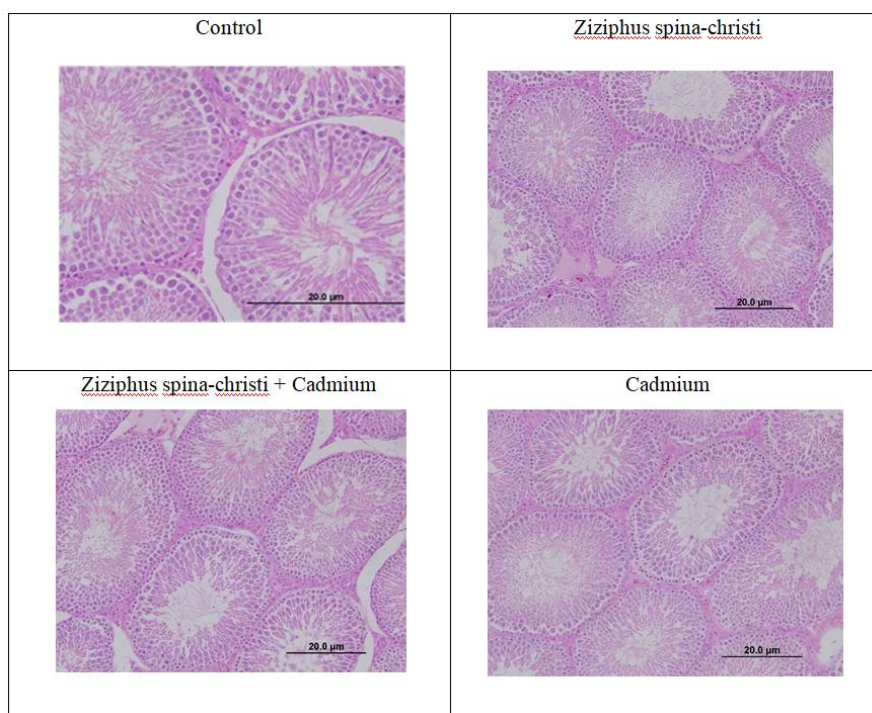


Figure 1. Histological analysis of ovarian tissue: effects of Cadmium and protective role of *Ziziphus spina-christi*.

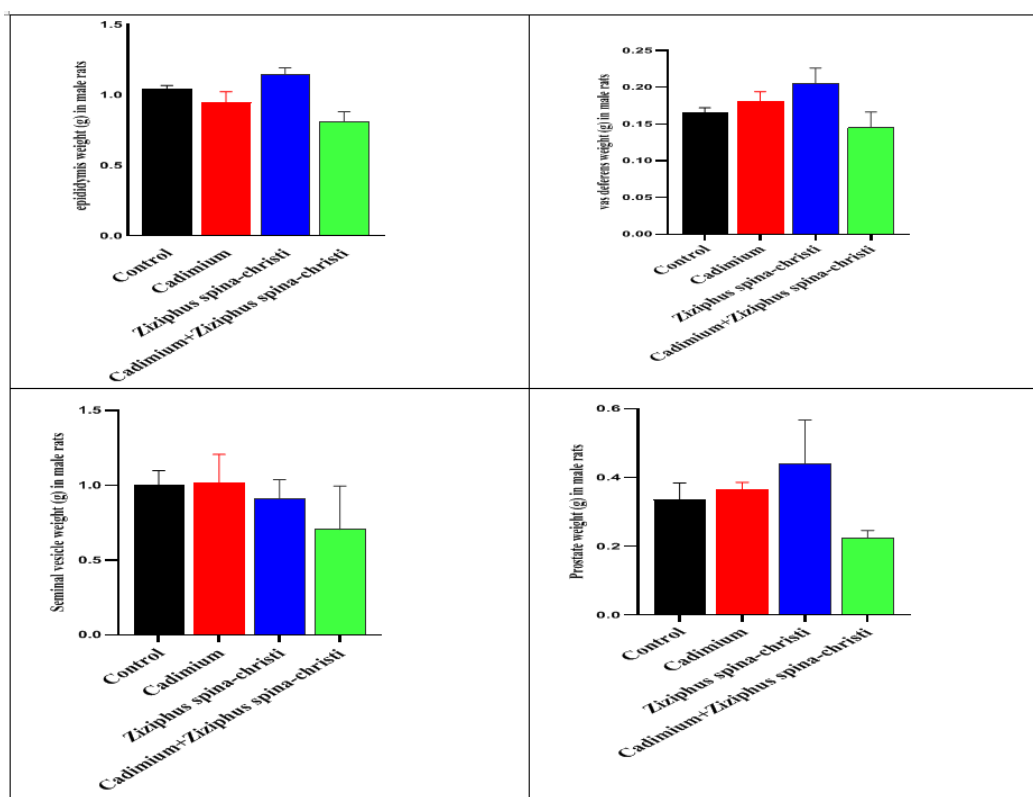


Figure 2. Effect of Cadmium and *Ziziphus spina-christi* on reproductive organ weights in male rats.

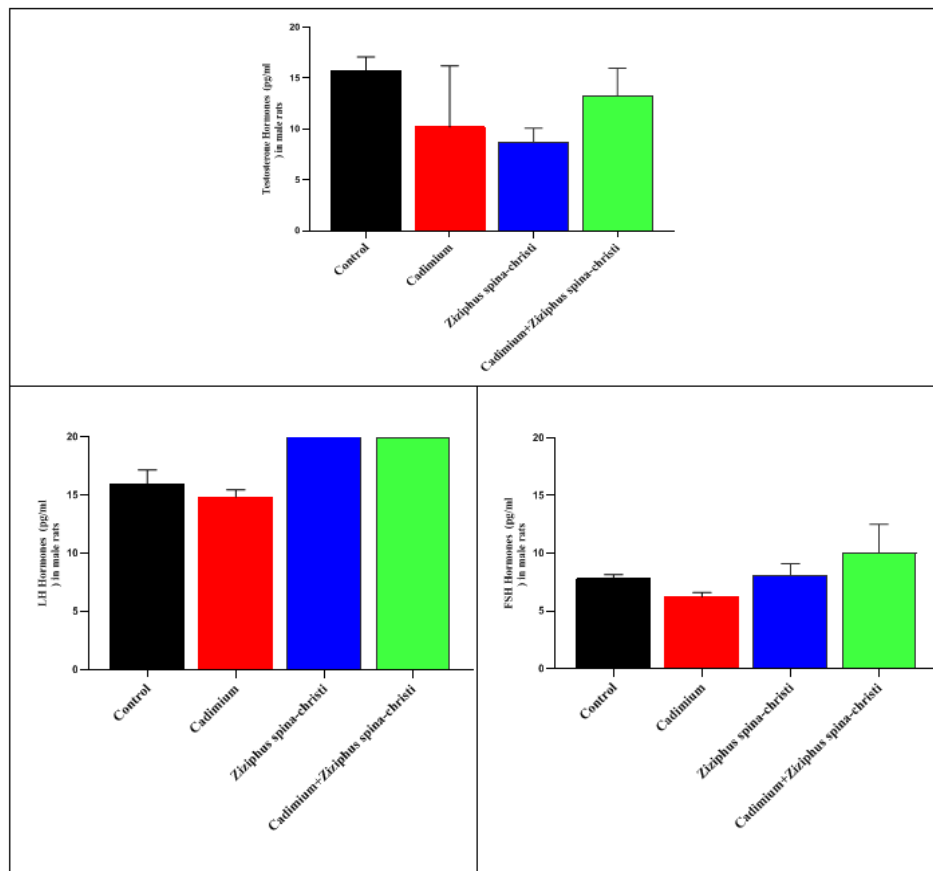


Figure 3. Impact of Cadmium and *Ziziphus spina-christi* on hormonal levels (Testosterone, FSH, LH) in male rats.

DISCUSSION

The bioactivity of *Ziziphus spina-christi* on cadmium-induced reproductive toxicity have been investigated and the results reveals a promising protective property of the plant against the detrimental effects of cadmium on male reproductive health. Cadmium is a heavy metal and as well a contaminant of emerging concern known to induce reproductive toxicity by disrupting hormone levels, impairing sperm production, and damaging reproductive organs. However, the administration of *Ziziphus spina-christi* has shown potential in mitigating these adverse effects. The weight of some of the male reproductive organs including the testes, epididymis, vas deferens, seminal vesicle and prostate gland were significantly altered by the exposure of cadmium. Cadmium exposure was previously reported to induce testicular atrophy and a significant decrease in testis weight due to its direct toxic effects on testicular tissue and

spermatogenesis (Iqbal *et al.*, 2021). Similarly, Cadmium was also reported to cause a reduction in epididymal weight and sperm count in rats exposed to cadmium, thus this is attributed to disrupted androgenic regulation and direct toxic impacts on the epididymis (Ali *et al.*, 2022). Also, the exposure of Cadmium in rat has been reported to cause deleterious effects in vas deferens, seminal vesicle and prostate gland that disrupts androgenic activity and atrophy in the accessory sex organs (Zhang *et al.*, 2021, Iqbal *et al.* 2021). The administration of *Ziziphus spina-christi* appears to mitigate cadmium induced decrease in weight of the male reproductive organs. The significant increase in the weight of these organs post treatment of Cadmium exposed rat with the plant extract can be attributed to its richness in vital phytochemicals that can impede the antioxidants and reactive oxygen species generation. Medicinal plants with antioxidant properties could protect against cadmium-induced testicular damage by reducing oxidative

stress and improving spermatogenic activity (Han *et al.*, 2020; Yu *et al.*, 2020; Li *et al.*, 2020). Furthermore, the results from the studies have implicated cadmium exposure to significantly reduces levels of crucial reproductive hormones, such as testosterone, Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH). The significant reduction in LH, FSH levels in cadmium exposed rats has been associated with cadmium interference with hypothalamic-pituitary-gonadal axis function that hinder the secretion of the hormones (Xiong *et al.*, 2021; Massányi *et al.*, 2020). In addition, the reduction in testosterone level after cadmium exposure can be attributed cadmium toxicity on Leydig cells (Yu *et al.*, 2020). However, the observed increases in the levels of LH, FSH, and testosterone after the co-treatment of cadmium and *Ziziphus spina-christi* in male rats suggests a potential stimulatory effect on pituitary function. Herbal plants of medicinal values have been reported to improve endocrine-modulating properties and protect against heavy metal-induced hormonal disruptions by enhancing antioxidant defenses and reducing oxidative stress in the pituitary gland (Yadav *et al.*, 2020; Mirkov *et al.*, 2020).

The sections obtained from the histological analysis show the cadmium-induced testicular damage. Significant pathological changes such as disorganization of seminiferous tubules and reduction in spermatogenic cells observed in the cadmium group. Similar histopathological changes in cadmium-exposed testis tissue were previously reported by Zhou *et al.* (2022). Meanwhile, the administration of *Ziziphus spina-christi* alone demonstrates no pathological changes, indicating a protective or neutral effect on testicular tissue. The co-treatment of cadmium with *Ziziphus spina-christi* demonstrates a mild pathological change, indicating a mitigating effect of *Ziziphus spina-christi* on cadmium-induced testicular damage. The mitigating effect of the crude extract can be associated with its antioxidant potency mitigating the Histological damage by reducing oxidative stress and enhancing cellular repair mechanisms (Unsal *et al.*, 2020).

CONCLUSION

In a nutshell, the results of this study highlight the detrimental effects of cadmium on male reproductive health, including significant reductions in organ weights, hormone levels, and severe histological damage. However, *Ziziphus spina-christi* demonstrates a promising protective activity against cadmium induced male reproductive toxicity in rat, owing to its antioxidant properties. These findings are supported by a body of recent literature demonstrating the protective effects of phytotherapeutic agents against heavy metal toxicity. Future research should focus on elucidating the precise mechanisms by which *Ziziphus spina-christi* exerts its protective effects and exploring its potential therapeutic applications in mitigating cadmium toxicity.

ACKNOWLEDGMENTS

The authors sincerely acknowledge the Researcher Support Project (RSP-2024R232) for funding this work at King Saud University, Riyadh, Saudi Arabia.

REFERENCES

- AMMARI, Aiman A., *et al.* The possible side effects of *Ziziphus spina-christi* extract on the liver, kidneys of female rats. *Indian Journal of Animal Research*, v.58.7 p.1221-1225. 2024,
- ALI, W.; MA, Y.; ZHU, J. *et al.* Mechanisms of cadmium-induced testicular injury: a risk to male fertility. *Cells*, v.11, p.3601, 2022.
- ANYANWU, B.O.; ORISAKWE, O.E. Current mechanistic perspectives on male reproductive toxicity induced by heavy metals. *J. Environ. Sci. Health C*, v.38, p.204-244, 2020.
- DHANALEKSHMI, U.M.; KHAN, S.A.; ALAM, T.; MASOODI, M.H. An insight into the phytochemistry, traditional uses, and pharmacology of *ziziphus spina-christi* (L) Willd. (Sidr): an edible wild plant of Arabian Peninsula. In: MASOODI, M.H.; REHMAN, M.U. (Eds.). *Edible plants in health and diseases: phytochemical and pharmacological properties*. Singapore: Springer Singapore, 2022. v.2, p.43-93.

- HAN, C.; ZHU, Y.; YANG, Z. *et al.* Protective effect of *Polygonatum sibiricum* against cadmium-induced testicular injury in mice through inhibiting oxidative stress and mitochondria-mediated apoptosis. *J. Ethnopharmacol.*, v.261, p.113060, 2020.
- HOCALOĞLU-ÖZYIĞIT, A.; GENÇ, B.N. Cadmium in plants, humans and the environment. *Front. Life Sci. Relat. Technol.*, v.1, p.12-21, 2020.
- IQBAL, T.; CAO, M.; ZHAO, Z. *et al.* Damage to the testicular structure of rats by acute oral exposure of cadmium. *Int. J. Environ. Res. Public Health*, v.18, p.6038, 2021.
- LI, J. Antioxidant-rich plants ameliorate cadmium-induced reproductive toxicity. *J. Med. Plants Res.*, v.14, p.245-252, 2020.
- MAHMOUD, A.E.D.; EL-MAGHRABI, N.; HOSNY, M.; FAWZY, M. Biogenic synthesis of reduced graphene oxide from *Ziziphus spina-christi* (Christ's thorn jujube) extracts for catalytic, antimicrobial, and antioxidant potentialities. *Environ. Sci. Pollut. Res.*, v.29, p.89772-89787, 2022.
- MASSÁNYI, P.; MASSÁNYI, M.; MADEDDU, R. *et al.* Effects of cadmium, lead, and mercury on the structure and function of reproductive organs. *Toxics*, v.8, p.94, 2020.
- MIRKOV, I.; STOJKOVIĆ, D.; ALEKSANDROV, A.P. *et al.* Plant extracts and isolated compounds reduce parameters of oxidative stress induced by heavy metals: an up-to-date review on animal studies. *Curr. Pharm. Design*, v.26, p.1799-1815, 2020.
- XIONG, L.; ZHOU, B.; LIU, H.; CAI, L. Comprehensive review of cadmium toxicity mechanisms in male reproduction and therapeutic strategies. *Rev. Environ. Contam. Toxicol.*, v.258, p.151-193, 2021.
- YADAV, K.; GHADGE, P.; LANGEH, A. *et al.* A review on herbal medicinal plant for treatment of polycystic ovarian syndrome (PCOS). *Asian J. Pharm. Res. Dev.*, v.8, p.83-87, 2020.
- YU, W.; XU, Z.; GAO, Q. *et al.* Protective role of wogonin against cadmium induced testicular toxicity: involvement of antioxidant, anti-inflammatory and anti-apoptotic pathways. *Life Sci.*, v.258, p.118192, 2020.
- ZHANG, H. Cadmium disrupts androgenic activity and leads to atrophy of accessory sex organs. *Environ. Toxicol. Pharmacol.*, v.87, p.103684, 2021.
- ZHOU, J.; ZENG, L.; ZHANG, Y. *et al.* Cadmium exposure induces pyroptosis in testicular tissue by increasing oxidative stress and activating the AIM2 inflammasome pathway. *Sci. Total Environ.*, v.847, p.157500, 2022.
- UNSAL, Velid, *et al.* The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review. *Advanced pharmaceutical bulletin*, v.10.2, 184, 2020.