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Protective Role of Antioxidants in Cadmium-induced Reproductive Toxicity

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ABSTRACT

Background: Studies on the role of oxidative stress in cadmium-induced reproductive toxicity have prompted numerous investigations into the use of antioxidants against reproductive toxicity. Natural compounds including curcumin and resveratrol have proven to be effective in mitigating oxidative damage. *Ziziphus spina-christi* (ZSC), is known for its abundance in antioxidant profile and has shown considerable protective effects against toxicity from heavy metals like cadmium. This current study seeks to uncover the mechanism behind which ZSC antioxidant properties protect against cadmium-induced reproductive toxicity.

Methods: ZSC extract was prepared by harvesting, drying and processing the plant's leaves. *In-vivo* experimental design was carried out, 24 rats were grouped in four groups: control group, ZSC treated group, Cadmium induced group and ZSC treated Cadmium induced group. To examine the oxidative stress, markers such as catalase, superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH) were measured. Statistical analysis was performed using ANOVA, followed by Tukey's multiple comparisons test to evaluate group differences.

Result: Cadmium exposure led to a significantly decrease in catalase and SOD activities, alongside an increase in MDA levels, indicating an increased oxidative stress. Treatment with ZSC effectively restored these parameters towards normal levels, demonstrating its potent antioxidant activity. Interestingly, Females responded slightly better than males, suggesting a possible gender-specific effects. The observed reduction in cortisol levels further confirmed ZSC's stress-reducing properties. These findings align with earlier studies on ZSC's antioxidant and protective properties.

Key words: Antioxidants, Cadmium, Gender differences, Oxidative stress, *Ziziphus spina-christi*.

INTRODUCTION

Cadmium (Cd), one of the environmental pollutant heavy metals is well recognized for its toxicity, particularly its detrimental effects on the reproductive system. According to the World Health Organization, infertility affects approximately 15% of reproductive-aged couples worldwide and environmental toxins, such as cadmium, are increasingly recognized as contributing factors (WHO, 2020). Understanding the role of antioxidants in counteracting cadmium toxicity could provide critical insights into novel therapeutic strategies to improve reproductive health, particularly in populations at risk of heavy metal exposure. Cadmium-induced reproductive toxicity is mediated via the generation of oxidative stress, basically, an imbalance between reactive oxygen species (ROS) and antioxidant defenses causes cellular damage. The reproductive toxicity of cadmium is largely driven by its ability to induce oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates. This oxidative stress can lead to cellular damage, particularly in reproductive tissues, resulting in impaired reproductive outcomes. Studies have shown that cadmium disrupts the antioxidant defense mechanisms within reproductive organs, thereby increasing lipid peroxidation, damaging DNA and altering gene expression related to fertility (Cuypers *et al*., 2010; Angelis *et al*., 2017). Meanwhile, cadmium exposure for a long time has been attributed to disruption of hormone regulation, DNA damage and impaired gametogenesis, ultimately compromising fertility. However, numerous studies have reported the role of antioxidants as a promising intervention to mitigate the harmful effects of cadmium-induced reproductive toxicity. Antioxidants scavenge the ROS and help in maintaining cellular homeostasis. For instance, antioxidants from both natural and synthetic products library have shown protective properties in various models of cadmium-induced reproductive toxicity. Antioxidants such as Vitamin C, Vitamin E, N-acetylcysteine and melatonin have been shown to alleviate oxidative stress in various biological systems, including the reproductive organs (Adedokun, Bhat *et al*., 2024, El-Poli *et al*., 2022; Unsal *et al*., 2020). By restoring antioxidant defenses, these compounds can potentially mitigate cadmium's harmful

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effects on reproductive health. Curcumin, a natural polyphenol, has been reported to protect against cadmium-induced ovarian toxicity by enhancing the antioxidant defense system and reducing oxidative stress markers in rats (Sakr *et al*., 2018). Similarly, resveratrol, another potent antioxidant, has been shown to ameliorate cadmium-induced histopathological alterations and hormonal imbalances in female rats by modulating oxidative stress and inflammatory responses (Elgawish and Abdelrazek, 2014).

ZSC contains antioxidant secondary metabolites that have demonstrated potent protective bioactivity against oxidative stress-induced cellular damage. ZSC richness in flavonoids, saponins and polyphenols has been attributed to its its robust antioxidant properties and its specific protective effects against heavy metal toxicity. Plants secondary metabolites are multifaceted in their action, though the protective properties of ZSC is central to its antioxidants content scavenging the ROS. Studies have shown that ZSC extract significantly ameliorate oxidative stress markers and histopathological damage in the liver and kidneys of rats exposed to cadmium (Al-attar, 2011). Similarly, ZSC ameliorated lead-induced oxidative damage in rat tissues, suggesting its potential protective role against other heavy metals, including cadmium (Al-Jassabi *et al*., 2012). There are reports on its anti-inflammatory effects associated with heavy metal toxicity (Fan *et al*., 2023). Similarly, ZSC bioactive metabolites can modulate signaling pathways involved in apoptosis and cell survival, thereby preserving ovarian and uterine cellular integrity. For instance, flavonoids in *ZSC* have been shown to inhibit the activation of pro-apoptotic proteins and enhance the expression of anti-apoptotic proteins, protecting cells from oxidative stress-induced apoptosis (Hosseinzadeh *et al*., 2013).

Understanding the effects of cadmium on female reproductive health and identifying protective agents like ZSC is crucial for developing effective strategies to mitigate reproductive toxicity. This research aims to provide valuable insights into the protective role of *Ziziphus spina-christi*, contributing solution to the growing prevalence of infertility and reproductive diseases especially in area of high exposure to cadmium.

MATERIALS AND METHODS

Preparation of the *Ziziphus spina-christi* extract

Samples from the leaves of ZSC were collected in Dammam region (26.43°N 50.1°E) during October to December 2023. The samples were cleaned thoroughly and stored in a dark, sanitary environment until they were completely dried. Post drying, the leaves were ground into a fine powder. This powdered ZSC was then soaked in water for 24 hours and the resulting solution was filtered through Whatman paper. Following maceration, the extract was evaporated at temperatures between 50 and 60°C, after which it was frozen at -20°C until needed. The extract was prepared at a dosage concentration of 100 mg per kg of body weight dissolved in water (Ammari *et al*., 2024).

Experimental animals

The study was conducted on 24 healthy rats, equally divided into male and female groups (12 of each), with body weights ranging from 180 to 220 grams and ages between 12 and 14 weeks. These animals were obtained from the Zoology Department's animal facility at the college Science, King Saud University (KSU). The rats were housed in a well-ventilated environment at a controlled temperature of 25±2°C, under a 12-hour light-dark cycle. They were provided with a standard diet and had access to water throughout the experiment. All procedures were carried out in compliance with the ethics committee guidelines and Institutional Animal Care standards at KSU (Approval no: KSU-SE-23-06). The rats were divided into four groups, each consisting of six animals (three males and three females). The control group received water, the second group was administered 100 mg of ZSC, the third group received cadmium at a dose of 1 mg/kg and the fourth group was treated with both ZSC and cadmium for a period of 21 days.

Oxidative stress examinations

After weighing the livers from each group of rats, the tissues were immediately homogenized in phosphate-buffered saline (PBS) with a pH above 7.4. The homogenates were then centrifuged at 5000 g for 15 minutes at 4°C. The supernatant was collected, with 10% of the total volume used for measuring oxidative stress markers, including glutathione (GSH) and malondialdehyde (MDA), along with other antioxidants present in the liver.

Statistical analysis

All statistical analyses for the experiment were performed using GraphPad Prism software (version 10.1.1). The Shapiro-Wilk test was employed to ensure normal distribution of the data. A two-way ANOVA was used to analyze animal body weights, considering both day and extract variables, followed by Tukey's multiple comparison test. Hormone levels and reproductive organ weights were analyzed using a one-way ANOVA, also followed by Tukey's test for post-hoc analysis. The results are expressed as mean values with standard deviations and a p-value of 0.05 or lower was considered statistically significant.

RESULTS AND DISCUSSION

Oxidative stress plays a crucial role in the pathogenesis of various diseases. This is often exacerbated by toxic substances such as Cadmium (Cd). Antioxidant defenses, such as catalase, superoxide dismutase (SOD) and glutathione (GSH), counteract oxidative damage, while elevated levels of malondialdehyde (MDA) and cortisol indicate increased oxidative stress and physiological stress, respectively. This study investigates the impact of different treatments on antioxidant levels and stress markers in

both male and female rats. The treatments include a control group, cadmium (Cd) exposure, ZSC administration and a combination of cadmium with ZSC. The study measures key parameters including catalase, superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH) and cortisol levels. In the examination of catalase activity, we observed a similar baseline catalase activity in both sexes in the control group. In Cadmium exposed group, a significantly reduced catalase activity was observed in both sexes while males show a slightly greater decrease. The ZSC treatment group demonstrated an increased catalase levels in both genders, while females experiencing a slightly higher improvement. Interestingly, the treatment of ZSC with cadmium exposed group restored catalase activity, while females show a stronger response (Fig 1). Catalase is crucial for detoxifying hydrogen peroxide, a significant decrease in catalase activity in rats exposed to cadmium has been reported by Choudhary and Singh, 2000. However, the reports of Saied *et al*. (2008) support our findings on ZSC potency to improve the catalase activity. The slight gender difference observed may be due to hormonal influences, as estrogen has been shown to upregulate antioxidant enzymes (Chainy and Sahoo, 2020).

Similarly, cadmium exposure in rats is reported to have a significant decrease in SOD activity in cadmium exposed rats (Martinez Dominguez *et al*., 2010). Our results show a baseline SOD levels in the two sexes for the control group, meanwhile, Cadmium exposure caused a notable reduction in SOD activity in both sexes, while the males have a more reduced SOD level. Treatment with ZSC increased SOD levels for both genders, while females showing a slightly higher level. However, the treatment of the Cadmium exposed group with ZSC demonstrates an improved SOD activity and again, the female rats demonstrate a more significant improvement (Fig 1). Asgarpanah and Haghighat (2012) reports on the protective effects of ZSC against oxidative stress and its potent enhancement of SOD activity. The gender differences in SOD response may be linked to varying expression levels influenced by sex hormones, as reported by Marra *et al*. (2002). Also, the significant reduction in SOD level in male rats can be attributed to the fact that cadmium exposure posed

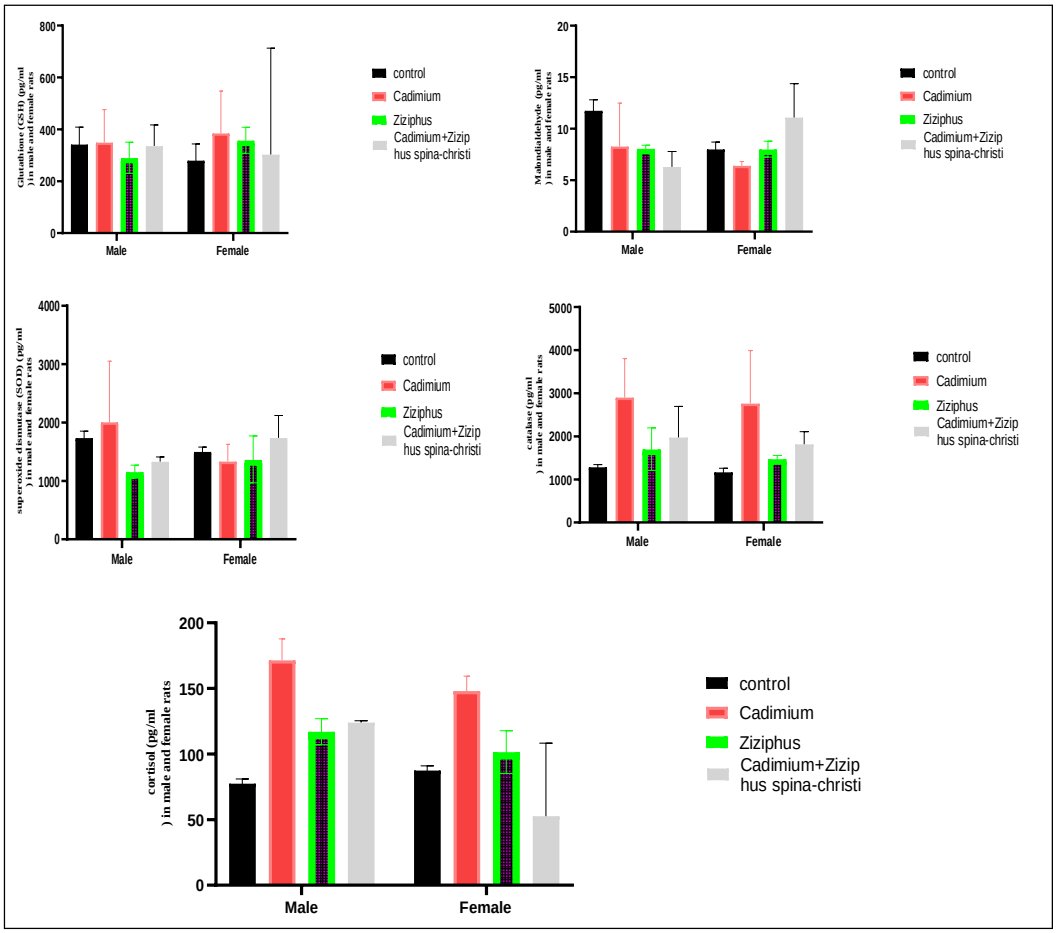

Fig 1: Impact of *Ziziphus spina-christi* on antioxidant enzyme activities and stress markers in male and female rats exposed to cadmium.

excessive superoxide anions, overwhelming the antioxidant defense mechanisms and more effects in the reproductive tissues of male rats (Makwana *et al*., 2020).

On the other hand, Increased MDA level indicates lipid peroxidation and oxidative stress. Our findings are consistent with that of Stacey *et al*. (1980) on elevated MDA levels in cadmium-exposed rats. Our results show a similar baseline MDA level in the control group for both sexes while the Cadmium exposure caused a significant increase in MDA levels, while male rats demonstrate a slightly greater increase. However, ZSC treatments in both Cadmium unexposed and exposed groups show a reduced MDA levels in both sexes, with females showing a slightly and stronger response respectively (Fig 1). ZSC's antioxidant properties due to its moderation of MDA levels and (Yossef *et al*. 2011). In addition, the slightly greater reduction in females could be linked to more effective lipid metabolism and antioxidant defense (Marra *et al*. 2002).

Furtherly, GSH is a critical antioxidant marker and its reduction by cadmium is well reported (Xu *et al*., 2003). Our studies show the baseline level for GSH is comparable in both sexes in the control group. Cadmium exposure caused a significant drop in GSH levels, with an increased response in males. ZSC treatment increased GSH levels, with females experiencing a slightly greater improvement. The treatment of cadmium exposed group with ZSC significantly restored GSH levels, with females showing a slightly better antioxidative response (Fig 1). The slightly better response in females might be due to enhanced GSH synthesis and recycling, influenced by hormonal differences (Chainy and Sahoo, 2020). The examination of cortisol also shows that both sexes had similar baseline cortisol levels in the control group. Cadmium exposure elevated cortisol levels, more significantly in males. ZSC treatment reduced cortisol levels in both genders, with females showing a marginally larger reduction. In correlation with other antioxidant markers testes, the treatment of Cadmium exposed group with ZSC significantly decreased cortisol levels, with females showing a stronger stress-reducing effect.

In general, our results demonstrate that ZSC exhibits a protective antioxidative effect against cadmium-induced toxicity in both male and female rats. In particular: Females consistently showed slightly better recovery in antioxidant enzyme activities and stress markers, suggesting potential gender differences in response to oxidative stress and antioxidative treatment. The significant restoration of catalase, SOD, GSH levels and the reduction in MDA and cortisol levels emphasize the importance of ZSC as a promising therapeutic agent to mitigate oxidative damage and reproductive toxicity caused by cadmium.

CONCLUSION

ZSC shows promise as a protective agent against cadmium induced oxidative stress. The slightly better response in females suggests potential gender-specific benefits warrants further investigation. In all, these findings support the therapeutic potential of ZSC in managing oxidative stress and its associated health effects.

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Data availability

The data sets used in the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors claim that there are no conflicting interests.

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