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Effects of Cadmium Exposure on Female Reproductive Health and the Protective Role of *Ziziphus spina-christi* in Rats

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ABSTRACT

Background: Long-term exposure to cadmium, a lasting environmental pollutant, has been associated with numerous negative health effects, particularly significant reproductive toxicity. This study examines the effects of cadmium on female reproductive health and to investigate the potential protective benefits of *Ziziphus spina-christi*, a medicinal plant with wide reports on its antioxidant properties, in reducing these impacts in rats.

Methods: The study was conducted using *in-vivo* model, sixteen female rats were assigned to four groups: Control, cadmium exposed group, *Ziziphus spina-christi* treated group and cadmium exposed treated with *Ziziphus spina-christi*. All treatments were conducted for 21 days. Histopathological assessments of ovarian tissues were carried out, along with measurements of organ weights and serum hormone levels (LH, FSH and estrogen) using ELISA kits. Statistical analyses were conducted with GraphPad Prism.

Result: Exposure of Cadmium in female rats significantly reduced ovarian, oviduct and uterus weights, indicating detrimental effects on reproductive organs. Histological analysis revealed degeneration, atrophy, increased fibrosis and inflammatory cell infiltration in tissues exposed to cadmium. In contrast, treatment with *Ziziphus spina-christi* maintained normal tissue structure and organ weights. The treatment of Cadmium exposed group with *Ziziphus spina-christi* displayed intermediate characteristics, indicating some protective effect. Hormonal analysis showed significant decreases in LH, FSH and estrogen levels due to cadmium exposure, while *Ziziphus spina-christi* treatment raised these hormone levels, suggesting a protective role against hormone disruption. These findings underscore the severe effects of cadmium on female reproductive health and highlight the potential of *Ziziphus spina-christi* as a therapeutic agent against cadmium-induced reproductive toxicity, warranting further investigation into its mechanisms and effectiveness.

Key words: Antioxidants, Cadmium, Oxidative stress, Rats, Reproductive health, *Ziziphus spina-christi*.

INTRODUCTION

Heavy metals including Cadmium are known environmental pollutants with significant health risks. In particular, they have pose negative impacts on the female reproductive system. Studies have reported that chronic exposure to cadmium is associated to an impaired ovarian function, hormonal imbalances, oxidative stress and infertility In addition, the disruption caused to the reproductive endocrine system can lead to structural damage in the ovaries and uterus and ultimately result in adverse pregnancy outcomes (Genchi *et al*., 2020; Hu *et al*., 2023; Kulsum *et al*., 2023; Arain *et al*., 2024; Zuhra, 2024; Knight, 2024; Edem *et al*., 2024; Ibadi *et al*., 2024; Bhat *et al*., 2024; Waseem *et al*., 2024). Cadmium is a persistent environmental pollutant that accumulates in several organs, including the liver, kidneys and reproductive tissues. It disrupts reproductive functions through mechanisms like oxidative stress, endocrine disruption and direct damage to ovarian and uterine tissues. Studies have shown that cadmium exposure can result in decreased fertility, ovarian dysfunction and histopathological changes in reproductive organs. For instance, cadmium disrupts the hypothalamic-pituitary-gonadal axis, leading to hormonal imbalances that negatively impact reproductive health (Vigeh *et al*., 2011). Cadmium exposure can also disrupt zinc and copper homeostasis in ovarian cells, leading to impaired steroidogenesis and ovarian dysfunction (Benoff *et al*., 2000). In addition, cadmium exposure in rats resulted in significant histopathological changes in ovarian tissues, including follicular atresia and degeneration, coupled with reduced serum levels of essential reproductive hormones such as estrogen and progesterone (Liu *et al*., 2015). On the other hand, the antioxidants from natural products library are potent in mitigating the reproductive toxicity effects of cadmium. Plants of medicinal values are repository for the antioxidant and many other potent phytochemicals that have been used since the ancient age. *Ziziphus spina-Christi* also

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known as Christ's Thorn Jujube is a well-known medicinal plant native to the Middle East, North Africa and parts of Asia. Its use in traditional medicine include as antioxidant, anti-inflammatory, antimicrobial and hepatoprotective effects due to its rich phytochemical profiles. In this regard, *Ziziphus spina-Christi* may offer protective benefits against cadmium-induced reproductive damage. Studies on animal models, particularly rats, suggest that the plant could counteract oxidative stress, restore hormonal balance and preserve ovarian function, providing a potential therapeutic avenue for mitigating cadmium toxicity in female reproductive health. Studies have highlighted the plant's ability to reduce oxidative stress and bolster cellular antioxidant defenses through its significant free radical scavenging activity in rat model (El-Mahmoudy *et al*., 2013). This study hypothesized that *Ziziphus spina-christi* can protect against cadmium-induced reproductive toxicity in female rats. We seek to evaluate the extent of reproductive damage caused by cadmium exposure and assess the antioxidant and protective effects of *Ziziphus spina-christi*.

MATERIALS AND METHODS

Plant collection and extraction

Leaves of *Ziziphus spina-christi* were collected in the Dammam region (26.43°N 50.1°E), between October and December 2023. After harvesting, the leaves were cleaned and stored in a dark, clean environment until fully dried. Once dried, they were ground into a fine powder. The powdered leaves were then soaked in water for 24 hours, followed by filtration using Whatman paper. The extract was macerated and evaporated at temperatures between 50 and 60°C, after which it was frozen at -20°C until dosage preparation, using a concentration of 100 mg/kg of body weight (Ammari *et al*., 2024).

Chemicals

Unless otherwise specified, all chemicals utilized in this undertaking were acquired from Sigma.

Chemical analysis using (GC-MS)

The volatile components of bile were analyzed using a Trace Ultra Gas Chromatograph paired with a DSQII Mass Spectrometer (Thermo Scientific). The components were separated using a TR-5 MS capillary column. This column was 30 meters long, with an inner diameter of 0.25 mm and a film thickness of 0.25 μm. Compound identification was achieved by referencing literature and equipment databases, including Adams Book 07, Nist 98 and Xcalibur. The Relative Retention Index was calculated using a series of n-alkanes ranging from C8 to C24. The relative percentages of the compounds were determined electronically from the percentage area data.

In-vivo experimental design

Healthy female rats were sourced from the animal facility at the Zoology Department, Science College, King Saud University (KSU). The rats were acclimatized in a well-ventilated environment with a stable room temperature of 25±2°C, maintained under a regular 12-hour light/dark cycle and given a standard diet with access to water. All experimental procedures adhered to the ethical guidelines approved by the KSU ethics committee (Approval no: KSU-SE-23-6) and Institutional Animal Care at KSU. The study included twelve female rats, weighed between 200-240 grams and aged 12-15 weeks. They were divided into four groups, each consisting of three rats: The control group (G-control) received no treatment; the G-1 mg/kg group was administered cadmium; the G-100 mg/kg group received *Ziziphus spina-Christi* extract; and the G-Cad+Ziz group was treated with both cadmium and *Ziziphus spina-Christi* extract for 21 days.

Biochemical analysis

Blood samples were collected from the hearts of the animals into non-heparinized tubes on the 15th day of the treatment. The samples were cooled at approximately 4°C overnight and then centrifuged at 1000x g for 15 minutes to separate the serum, which was subsequently frozen at -20°C for hormonal analysis. Hormone concentrations in the serum samples were measured using the enzyme-linked immunoassay (ELISA) method, employing ELISA kits from MOLEQULE-ON (Auckland, New Zealand) namely, 17 β-estradiol (Cat No. ELI-M-036-96) and Progesterone (Cat No. ELI-M-034-96).

Histomorphometric examination

Ovaries of the experimented rats were surgically removed under CO2 anesthesia. The harvested ovaries were immersed in 10% neutral formalin for 48 hours for fixation. The fixed gonads were dehydrated through a series of alcohol solutions, infiltrated with xylene for 4-6 hours, embedded in paraffin wax cubes, sectioned with a microtome and mounted on slides before being stained with hematoxylin and eosin (H and E). Histomorphological and histomorphometric evaluations were performed using a light microscope. Ovarian follicles were counted and classified across all animal groups following the method described by Adelakun *et al*. (2022), with slight modifications. Primordial follicles were identified by a patchy layer of squamous cells surrounding the oocyte. Primary and secondary follicles were recognized by the presence of one row and two rows of cuboidal cells, respectively. Tertiary follicles were characterized by multiple layers and a small antrum, while Graafian follicles were distinguished by a large follicular antrum.

Statistical analysis

All statistical analyses from this experiment were performed using the GraphPad Prism (10.1.0) software. The Shapiro-Wilk test confirmed that the data followed a normal distribution. Body weights of the animals were analyzed using two-way ANOVA (considering day and extract factors) and Tukey's multiple comparisons test. Hormone levels and 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 AND DISCUSSION

Ziziphus spina-christi ameliorates cadmium induced toxicity and increases organ function

The high antioxidant properties of *Ziziphus spina-christi* has been linked to ameliorative tendency of cadmium-induced toxicity (El-Beltagy *et al*., 2019) Exposure of cadmium in rat has led to the generation of oxidative stress, cellular damage and organ dysfunction. The antioxidant properties of Ziziphus spina-christi has been reported to reduce the toxicity and protect reproductive organs from cadmium-induced damage (Zhao *et al*., 2023). The result available from this study shows that, treatment of *Ziziphus spina-christi* in rats preserved the weights of reproductive organs when compared to control levels. In the cadmium + *Ziziphus spina-christi* group, the treatment presents a partial recovery of organ weights compared to the cadmium-only group, this suggests that *Ziziphus spina-christi* can mitigate the negative effects of cadmium. Fig 1 shows that in the control group, the ovarian tissue appears normal, exhibiting healthy follicles at various developmental stages, with no significant inflammatory cells or structural abnormalities. In contrast, the cadmium group shows signs of degeneration and atrophy, with a reduced number of healthy follicles, some of which are undergoing atresia. The stroma exhibits increased fibrosis and inflammatory cell infiltration. The *Ziziphus spina-christi* group displays a healthy structure similar to the control group, with follicles in different developmental stages and no notable signs of atresia or degeneration. Meanwhile, the *Ziziphus spina-christi* + cadmium group demonstrates characteristics that are intermediate between the cadmium-only group and the control. It shows some signs of follicular atresia and degeneration, but these effects are less severe than those in the cadmium-only group, with a less fibrotic stroma and fewer inflammatory cells present.

Similarly, the morphometric analysis of reproductive organs shows the effects of cadmium exposure and *Ziziphus spina-christi* treatment on the weights of reproductive organs (ovary, oviduct and uterus) in female rats. Fig 2 shows that cadmium exposure significantly reduced the weights of these organs compared to the control group, indicating damage to reproductive development and function. Specifically, cadmium decreased ovary weight from 0.5 g to 0.1 g, oviduct weight from 0.25 g to 0.1 g and uterus weight from 1 g to 0.2 g. Cadmium exposure has been reported to significantly reduced the weights of the ovary, oviduct and uterus, reflecting its detrimental impact on reproductive organ development and function (Pollack *et al*., 2014). However, treatment with *Ziziphus spina-christi* showed a protective effect, partially restoring organ weights when co-administered with cadmium. For example, ovary weight increased to 0.3 g, oviduct weight to 0.2 g and uterus weight to 0.5 g with combined treatment. Meanwhile, no significant difference between the control and *Ziziphus spina-christi* treated alone groups. These findings suggest that *Ziziphus*

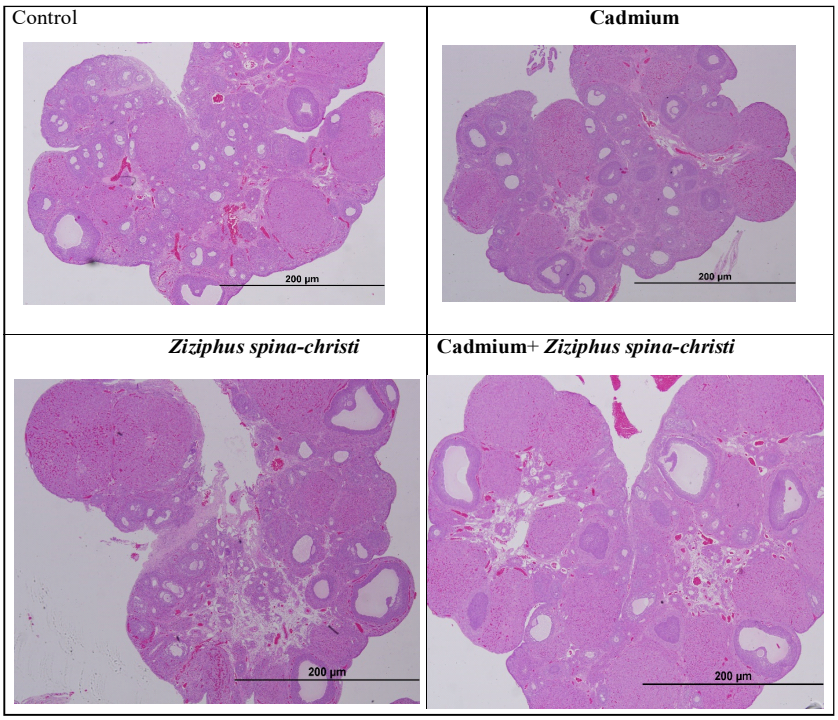

Fig 1: Histological analysis of reproductive organs in female rats exposed to cadmium and treated with *Ziziphus*.

spina-christi could be a potential therapeutic agent to mitigate cadmium-induced reproductive toxicity.

Ziziphus spina-christi increases and restores the reproductive hormonal level before and after cadmium exposure

Ziziphus spina-christi is a well-known plant for antioxidant and anti-inflammatory properties. Results indicate that the plant has potential in restoring reproductive hormone levels post cadmium exposure. Cadmium exposure significantly demonstrated a hormonal imbalance in rats. Basically, cadmium disrupts the endocrine system and eventually, hormones that are crucial for normal reproductive health become reduced in synthesis. This reduction in synthesis can impair fertility and other reproductive processes (McClam *et al*., 2022). As shown in Fig 3, the examination of key reproductive hormones including the Luteinizing

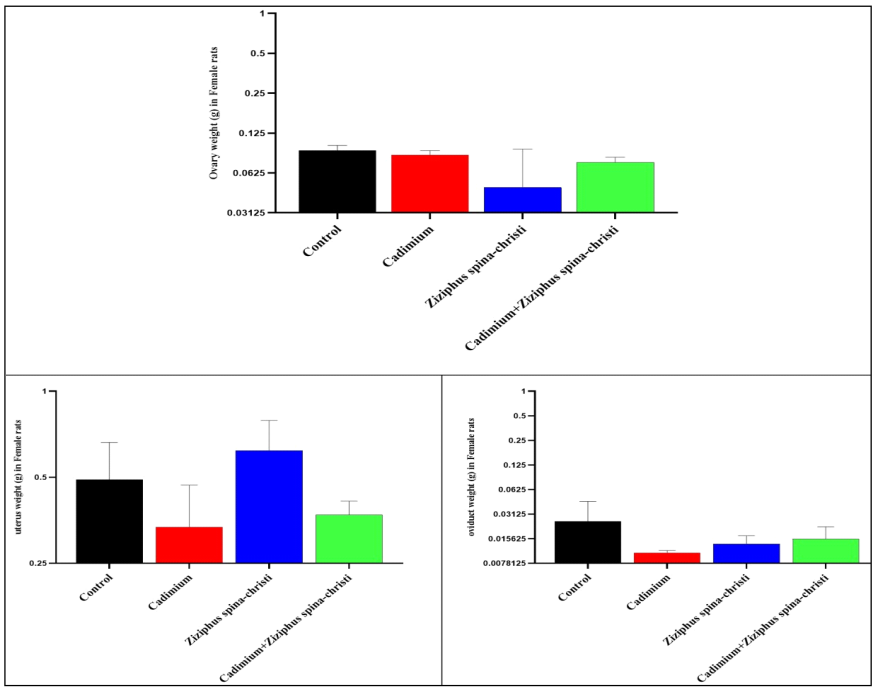

Fig 2: Impact of cadmium and *Ziziphus spina-christi* on reproductive organ weights in female rats

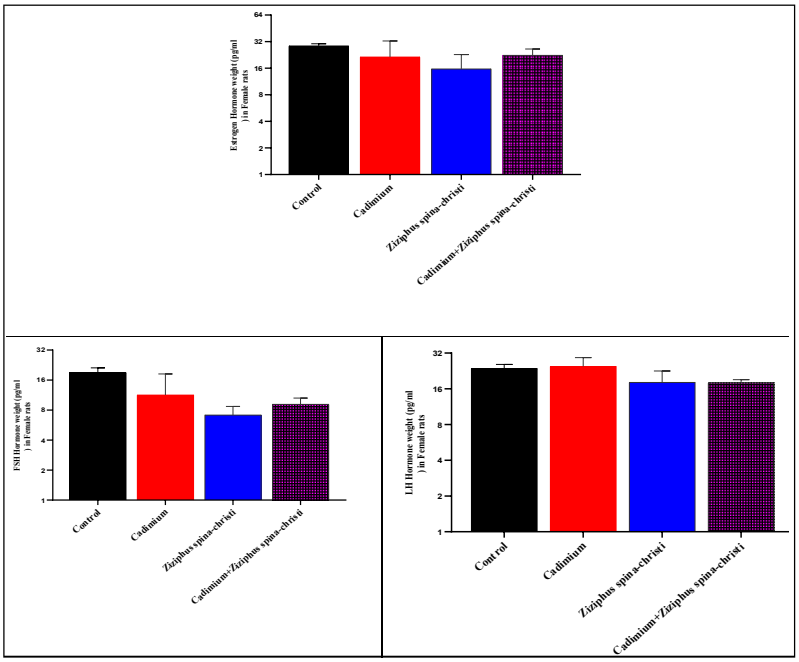

Fig 3: Effects of cadmium and *Ziziphus spina-christi* on hormone levels (LH, FSH and Estrogen) in female rats.

Hormone (LH), Follicle Stimulating Hormone (FSH) and estrogen shows that cadmium exposure significantly reduced LH (from 8 pg/ml to 3 pg/ml), FSH (from 16 pg/ml to 7 pg/ml) and estrogen (from 32 pg/ml to 12 pg/ml), indicating a negative impact on reproductive hormone regulation. The treatment with *Ziziphus spina-christi* increased hormone levels, both in the absence and presence of cadmium, showing a protective effect. Comparing to the control group, the levels of the 3 hormones under investigation were raised in *Ziziphus spina-christi* treated only group to 14 pg/ml, 18 pg/ml and 64 pg/ml for LH, FSH and Estrogen respectively. Co-treatment of cadmium and *Ziziphus spina-christi* when compared with the control group and Cadmium exposed group demonstrate a partial restoration of the hormones. The hormones levels were significantly increased to 7 pg/ml, 11 pg/ml and 24 pg/ml for ml for LH, FSH and Estrogen respectively. This highlights the plant's potential to mitigate cadmium toxicity. Previous studies have demonstrated that *Ziziphus spina-christi* treatment, either alone or in combination with cadmium, can partially restore reproductive hormone levels. For instance, cadmium-exposed rats showed marked reductions in LH, FSH and estrogen, but co-treatment with *Ziziphus spina-christi* significantly improved these levels, although not to control levels, indicating its protective role against cadmium-induced reproductive toxicity (Pollack *et al*., 2014; Fatima *et al*., 2019). This protective effect is likely due to the antioxidant properties of Ziziphus spina-christi, which can reduce oxidative stress and inflammation (McClam *et al*., 2023).

CONCLUSION

While current studies elaborate the promising evidence of *Ziziphus spina-christi's* protective effects against oxidative stress and heavy metal toxicity, further research is required to examine the specific mechanisms of action of the plant extract in addressing cadmium-induced reproductive toxicity. Future research should focus on molecular analyses, dose-response effects and the long-term impact of *Ziziphus spina-christi*. Clinical studies are also necessary to confirm these findings in humans and evaluate its therapeutic potential. This study highlights cadmium's harmful effects on female reproductive hormones and the beneficial role of **Ziziphus spina-christi** in mitigating these effects, suggesting its potential for future therapeutic use.

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Disclaimers

The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All experimental procedures followed the guidelines specified by the ethics committee (Approval no: KSU-SE-23-6) and the Institutional Animal Care at KSU.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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