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THE INVESTIGATION OF IMMUNOLOGICAL AND OXIDATIVE STRESS IN PROSTATE ISSUES OF RATE EXPOSED TO CADMIUM SULFATE AND EVALUATED OF PROTECTIVE/PROPHYLACTIC EFFECTS OF *TERFEZIA BOUDIERI* IN THE SAME TISSUES.

Alaa Abd-Alhasan Hamdan[1], Hassanin Noor Hadi Al-Mousawi [2], Mohammed Talat Abbas[3], Ayla Khedher Ghalib[4]

1 , 2 -Department of Laboratory sciences, College of pharmacy, University of Kerbala,Iraq
3. Department of Pharmaceutics, College of pharmacy, University of Kirkuk. Kirkuk, Iraq.
34Department of Gynecology & Obestrics, College of medicine, University of Kirkuk, Kirkuk, Iraq.

Abstract:

Objective: To assess the effectiveness of *Terfezia boudieri* extracts in protection against cadmium sulfate (CdSO4) induced toxicity in stimulating immunological and oxidative stress in male rats.

Methods: A total of 30 healthy adult rats (weighting 240-260g) were included in the current study. These rats were randomly distributed into 5 groups, each of 6 rats. The first group (control) was given no treatment for 8 weeks, and the second group was administered CdSO4 orally at 28 mg/kg/day for 15 days (experiment period). The third group received Terfezia *boudieri* extracts at 300 mg/kg intraperitoneally (IP) every day, the fourth group was given a combination of CdSO4 and Terfezia *boudieri* extracts at 300 mg/kg /day, and the fifth group was treated with *Terfezia boudrieri at* 300 mg/kg/ day intraperitoneally following by CdSO4.

ELISA was used to evaluate cytokine levels, and a VIDAS automated mini-analyzer was used to determine serum PSA activities. Prostate tissue Malondialdehyde level (MDA), prostate tissue activity, prostate tissue glutathione Peroxidase (GSHPX), Superoxide dismutase (SOD) activity, Catalase (CAT) and total protein were assessed. Oxidation of NADPH is monitored spectrophotometrically at 340 nm.

Results: Cadmium sulfate increased the production of IL-1β, serum PSA activity, prostate tissue MDA levels, and decreased levels of (IL-6, IL-10), (CAT), (SOD), and (GSHPX). The alteration of these biological parameters is significantly improved by using Terfezia boudrieri extract.

Conclusion: Protective and prophylactic effects of *Terfezia boudrieri* were observed compared to the toxic effects and deterioration of pro-inflammatory and anti-inflammatory cytokines after exposure to cadmium sulfate in the prostate gland.

Key words: Cadmium sulfate, Terfezia *boudrieri*, immunological and Oxidative stress, prostate, rats.

Introduction:

Generally, humans are exposed to the toxicity of cadmium sulfate in water, food, as well as smoke. Cadmium sulfate is not biodegradable like other organic compounds. Ongoing cadmium sulfate exposure causes nephrotoxicity, prostate necrosis, and osteoporosis. In addition, it causes neurodegenerative diseases, kidney failure, and infertility 1.

The immune system's function modulates with the accumulation of Cd in immune cells, triggering immunological responses, which cause different health problems. The immune cell's activity and apoptosis are affected by Cd, which considers an immunotoxic agent, modifying the immune cytokines secretion, stimulating the production of oxidative stress and ROS, altering the cloning of T lymphocyte subsets, and changing the make of special antibodies from specific immune cells 2.

Cells generate a range of (ROS); these compounds are naturally produced as a result of aerobic metabolism, by interactions with environmental toxins and drugs, and when the antioxidant level that activates it decreases, all leading to a state of oxidative stress (OS). High ROS can cause DNA chemical damage, proteins, and lipids, resulting in cell death. ROS has been involved in many pathological conditions, including cancer, inflammatory diseases, and aging 3.

Today, medicinal plants are increasingly being used to prevent and treat diseases. In some cases, folk medicine, especially pharmacognosy, is the perfect cure 4. Truffle (*Terfezia boudieri*) is one of the oldest food. Due to its delicious taste and musky aroma, it is consumed largely as a substitute to meat 5,6. It is naturally grown during autumn rains and thunderstorms in Turkey, Kuwait, Iran, and Iraq 7,8. Truffles (Terfezia boudieri) are rich in amino acids, proteins, fats, minerals, and carbohydrates 9,10. Furthermore, it contains phytosterols, terpenoids, phenols, and polysaccharides which contribute to its antibacterial, anti-inflammatory, antitumor, and antioxidant properties 10,11.

Materials and methods

Preparation of cadmium sulfate (CdSO4) doses

CdSO4 toxic dose was prepared by dissolving 28 mg in 1000 mL of distilled water. Then, the aqueous suspension was administered at a dose of (28 mg/kg) by oral gnawing12,13.

Preparation of Terfezia boudieri extract:

Terfezia boudieri were collected from different places in Kerbela province (Iraq). It was identified, characterized, and classified by Prof. Dr. Ibrehim Saleh (Department of Pharmacognosy, College of Pharmacy, University of Al-Mustansiria) Samples were cleaned in the laboratory, and all parts of Terfezia boudrieri were air dried in the dark, followed by grinding. Terfezia boudrieri extract was prepared by adding ethanol to 100 g of the dried plant, heating it in a Marie bath (45 °C for 24 h), and leaving the leaves at room temperature (1 h) . Afterward, a rotary evaporator in vacuo (60 r/min, 64 °C) was used to concentrate the extracts. As described in (11), Terfezia boudrieri extract was used at a dose of 300 mg/kg.

Experimental animals

A total of 30 adults healthy Wistar Albino male rats (240-260g) were obtained from the laboratory animal house, College of pharmacy, University of kerbala Animals were kept in standard cages, where the standard laboratory conditions were maintained (natural light/dark cycle, room temperature 22 ± 3°C). Animals were given a standard dry rat pellet diet, and tap water was

provided *ad libitum*. The University of Kerbala, College of Pharmacy – Scientific and Ethical Committee approved the experimental protocol NO. 2022N.1.

The rats were grouped into 5 groups, each of 6. Group 1 (Control) was given water and a standard diet for 8 weeks. Group 2 (CdSO4), were treated with 28 mg/kg/b.w cadmium sulfate (CdSO4) via gastric tube at fifteen days of the experiment. Group 3, (*Terfezia boudrieri)* were given (300 mg/kg/day) of *Terfezia boudrieri through* intraperitoneal injection(IP). Group 4 (Cadmium Sulfate (CdSO4)+ *Terfezia boudrieri* were given synchronized via gavage CdSO4 plus *Terfezia boudrieri* 300 mg/kg /day as an intraperitoneal injection at fifteen days of the experiment. Group 5 [(Cadmium Sulfate (CdSO4) + *Terfezia boudrieri* (Pre)] rats were given (300 mg/kg/day) with IP Terfezia boudrieri on the first day of the experiment, then received CdSO4 on the day 15th of the experiment.

At the end of the experiment, animals were sacrificed ,Blood samples were collected in centrifuge tubes; then, the serum was separated by centrifugation at 860g for 20 min. The serum was stored at (-20°C) for immunological and biochemical tests. Prostate was removed, washed with (%0.9) of cold NaCl solution, and stored at (-80°C.). one gram of prostate was homogenized with (4 ml %0.9 NaCl) at 4000 rpm before being centrifuged at 20000 rpm for 20 min. The supernatants were used for enzyme activity analyses (Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSHPX), and Malondialdehyde (MDA).

Immunological analysis:

Cytokine level was evaluated in all groups, serum levels of cytokines (IL-10, IL-1β, IL-6) were measured using the ELISA reader (at 450 nm), and the kits with all standards were bought from (Beijing Human. Diagnostics Company (double-antibody, sandwich ELISA, pg/mL). To confirm the statistical analysis, the data exceeds the minimum detectable concentrations (half of the minimum detectable concentrations) was considered. The hole was added as a blank control, and the samples whose OD value overridden the linear range should be diluted before detection. The experiment was conducted following the manufacturing protocol of the kit. The coefficient of variation (between plates and within plates) was less than 10%.

Biochemical analysis:

Serum PSA activities were determined by VIDAS automated mini-analyzer (BioMerieux, France). Prostate tissue Malondialdehyde level was estimated following the instructions (Uchiyama and Mihara) 14. (TBA) react with MDA in an acidic medium to make a pink compound, then spectrophotometrically metrical was read at (535 nm). Prostate tissue activity was evaluated following Beers and Cesar methods 15.

Catalase catalyzed the breaking down of hydrogen peroxide to H2O and oxygen, causing absorbance to decrease at (240 nm). Following the Winter Bourne methods, the SOD activity was determined 16. The prostate tissue glutathione Peroxidase was estimated following the instructions of Leopold Flone *et al.* method 17. The oxidized glutathione formed during a glutathione session is immediately and continuously reduced by increasing the activity of glutathione to a constant level of glutathione. Concomitant oxidation of NADPH is monitored spectrophotometrically at 340 nm. Lowery et al. method was followed to measure the total protein 18.

Statistical design:

The (SPSS 12) program was used to analyze the result. ANOVA and Tukey's tests were applied to compare different experimental animal sets. The results were illustrated as (means ± SD).

Results:

There was a significant increase in PSA levels in the rat group treated with CdSo4 compared with the control (Table 1). However, CdSo4 supplementation of intoxicated mice with Terfezia boudrieri attenuated the adverse effects of CdSo4 drugs, as indicated by the significant decrease in PSA levels. Also, there was a significant increase in IL-1β levels in the rat group treated with CdSo4 Compared with the control group. Nevertheless, the used dose of CdSo4 with *Terfezia boudrieri* improves the CdSo4 negative effects as proven by reduced IL-1β significantly (Table 2).

Administration of CdSo4 drugs (28 mg/kg/day) for 8 weeks led to increasing in the level of MDA while decreasing in the activities of antioxidant enzymes (SOD, CAT, and GHPXP) in the CdSo4 group as compared to the control group (Table 3). Intraperitoneal administration of *Terfezia boudrieri* (300 mg/kg/day) for 8 weeks causes a decrease in CdSo4 and preserves the male albino at normal condition.

Table 1: The effect of (CdSo4) on serum prostatic specific antigen (PSA).

Parameters	Control	CdSo4	*T. boudrieri*	CdSo4+ *T. boudrieri*	*T. boudrieri* CdSo4+
PSA (2x 10^{-2} ng/mL)	0.58±0.05	20.88±1.94*	0.54±0.07	0.62±0.09	0.58±0.06

Means ± SD, Significant differences (*P<0.05).

Table2: The effect of (CdSo4) on proinflammatory and anti-inflammatory cytokines.

Parameters	Control	CdSo4	*T. boudrieri*	CdSo4 + *T. boudrieri*	*T. boudrieri* + CdSo4
IL-1β (pg/ml)	8.8±5.3	13.7±3.2*	8.2±5.2	8.9±9.7	9.0±5.2
IL-6 (pg/ml)	3.68± 1.44	1.22 ±1.24*	3.81± 1.34	3.38± 1.64	3.21 ±1.34
IL-10 (pg/ml)	0.82±0.56	0.51±0.16*	0.91±0.56	0.72±0.56	0.72±0.11

Means ± SD , Significant differences (*P<0.05)

Table (3) : The effect of (CdSo4) on MDA and antioxidant enzymes.

Parameters	Control	CdSo4	*T. boudrieri*	CdSo4+ *T. boudrieri*	*T. boudrieri* CdSo4+
MDA (Nmol for each mg protein)	0.16±0.08	0.81±0.08*	0.15±0.007	0.16±0.01	0.16±0.08
SOD	9.35±1.23	3.35±0.48*	10.83±1.20	9.55±1.16	9.25±0.65

(U/mg protein)					
CAT (IU/mg protein)	0.90±0.12	0.27±0.04*	0.93±0.15	0.88±0.10	0.88±0.11
GSH_{PX} (IU/mg protein)	3.89±0.04	1.66±0.19*	3.98±0.10	3.87±0.12	3.78±0.15

Means ±SD, Significant differences(*P<0.05).

Discussion:

This study was conducted to determine the role of extraction of *Terfezia boudrieri* in reducing the adverse effects of cadmium sulfate in the production of oxidative stress, pro-inflammatory, and anti-inflammatory cytokines in male albino rats. The prostate function was evaluated by measuring serum prostatic specific antigen (SPA). Also, this study evaluated the oxidative stress in male rats by measuring the level of (MDA) levels (GSHPX), (CAT), and (SOD). There was a significant increase (P<0.05) in serum IL-1β (pg/ml) concentration and a significant decrease (P<0.05) in both serum IL-6 (pg/ml) and IL-10 (pg/ml) in CdSO4 treated groups as compared to control groups; these results agree with the study conducted by (19). The Cd effect has undesirably affected human health by reducing the efficiency of the immune system in dealing with infection and fighting cancer and urges the emergence of autoimmune diseases and allergies, even in small quantities of exposure 20. The immune system's function modulates with the accumulation of Cd in immune cells, then cause triggering for immunological responses, arise different health problems. Cd affects cell death and immune cell activity as an immunotoxic agent, modifies the immune cytokines secretion, stimulates the production of (ROS) and then causes oxidative stress, altering the cloning of T lymphocyte subsets and changing the manufacture of special immunoglobulin in plasma cell 21.

Cd, an immunotoxic inhibitor with certain concentrations, directly reacts with the immune system, alters the efficiency and function of specific cells, then causes damage to immunity in a dose-dependent manner upon frequency exposure 22. Cadmium alters the activity of immune cells and affects cytokine production by affecting the level of gene expression. It stimulates the expression of the pro-inflammatory factors in macrophages by inducing ROS production, on the other hand inhibiting the production of (anti-inflammatory cytokine) IL-10 23.

Serum PSA concentration increased significantly at (P<0.05) in CdSO4 treated groups compared to control groups. PSA is an important protein produced by the epithelial cells of the prostate and secreted within the spermatic fluid. It possesses an essential role in filtering sperm and detecting prostate diseases 24,25. Under normal conditions, SPA enters the circulation by extravasation from the cell and diffusion into the vein and capillaries 26. However, in animals exposed to CdSO4, the basal cell layer, and cell polarity change prostatic tissue growth disturbances. The secretion of SPA is directed and free prostate antigens are produced into the circulation, resulting in elevated PSA levels in animals exposed to CdSO4. However, administration of Terfezia boudrieri and CdSo4 improved prostate function, as indicated by the significant restoration of serum PSA activity. Our

results agree with some studies which recorded the rise of PSA in CdSo4 exposed animals 27.

There was a substantial increase at ($p < 0.05$) in MDA levels for the CdSO4-treated group as compared with the control. Induction of intraperitoneal oxidative stress by CdSo4 induces oxidative effects that oxidize unsaturated fatty acids in cell membrane lipids and ultimately lead to the production of a large dose of cytotoxic compounds, including MDA and oxidative stress. It may produce oxygen hydroxyl (OH•) radicals that are highly active in cracking prostate tissue, raising lipid peroxidation levels and MDA. MDA is the final output of the LPO process, and the estimation level of MDA Gives a good rating of LPO, which is among the main mechanisms of cell damage leading to necrosis or apoptosis 28.

On the other hand, there was a significant decrease ($P < 0.05$) in SOD, CAT, and GSH-px activity in CdSo4-treated prostate tissue. This is because CdSo4 generates free radicals, which disturb the arrangement of antioxidants and eventually cause oxidative stress; this outcome is consistent with other studies 28,29. However, administration of the extract (Terfezia boudrieri) accompanied by CdSo4 led to a decrease in the level of MDA and also increasing in SOD, CAT, and GSH-px activity in prostate tissue compared to the control group; this outcome may be proven in the protectionist role of extraction (Terfezia boudrieri). The protective role of (Terfezia boudrieri) can be attributed to the scavenging function of free radicals and being an important source of flavonoids 29. Polyphenol compounds characterized by their ability to safeguard the cell from oxidation are also considered antioxidants 30. some other studies highlighted the ability of extraction (Terfezia boudrieri) to act as an antioxidant to the fact that it contains many vitamins and chemical compounds such as (A, B-carotenoids, C, and many phenolic compounds) 31.

Study limitations. Following the Guidelines of laboratory animals use and regulations of Kerbala University, it was not feasible to test more laboratory animals as per The European Council Directive recommendations (2010/63/EU), which are based on the principle of Three Rs, replace, reduce, and refine the use of animals used in scientific research. The authors must conduct their study using a sample size of 30 mice. The sample size could be increased in future studies to have better insight into the efficacy of Terfezia boudrieri. This study is necessary to suggest further studies that could help heighten the medical importance of Terfezia boudrieri.

Prospects for further studies. Further studies are required, including conducting a well-controlled study with a larger sample size to determine the therapeutic effect of Terfezia boudrieri in vivo.

Conclusion

We conclude from this study that exposing male mice to CdSo4 increases the levels of LPO, but the administration of (Terfezia boudrieri) extract to animals exposed to CdSo4 reduces the levels of MDA and improves the effectiveness of CAT, SOD, and GSHPX in prostate tissue. Based on the obtained results, Terfezia boudrieri may have a potential protective effect against oxidative injury in the prostate.

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