Research Article

The Protective Effects of Olive (*Olea oleaster*) Leaves Extract on Male Rats Exposed to Cadmium Toxicity

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Abstract | Environmental pollution is an issue of complex dimensions and getting much interest from researchers and governments. Globally, the environment is suffering from the increased rates of pollution that seriously threaten the stability of the ecosystem and living organisms. Currently, the environment is increasingly exposed to chemical pollutants, including heavy metals, which affect humans, animals and plants. Cadmium (Cd) is a heavy metal known as a toxic factor to humans, animals and plants. Saudi Arabia is enriched with medicinal plants whose therapeutic effects are not sufficiently known and not exploited properly. Medicinal plants play an important role in treatment of human health problems since past decades until now. The present study might be the first trial to examine the effects of olive (*Olea oleaster*) leaves extract on male rats exposed to Cd (15 mg/kg body weight (b.w.). The male rats were randomly assigned to four groups. The first group served as control (CTL) that orally administered saline solution. The second group was exposed to Cd in a dose of 15 mg/kg b.w. The third group was treated with olive leaves extract (600 mg/kg b.w.) and exposed to Cd. The fourth group was treated with olive leaves extract (600 mg/kg b.w.) only. At the end of the experimental period, blood samples were collected and analyzed. The obtained results showed that the levels of selected blood biochemical parameters (ALT, AST, ALP, total bilirubin, creatinine (Cr), BUN, uric acid, MDA, IL-6 and TNF-α) were significantly increased in rats of the second group. Moreover, the levels of total protein, albumin and CAT were statistically declined. The *O. oleaster* leaves' extract could nearly normalize those blood parameters for protection against Cd toxicity. It might be concluded from this study that the protective effect of *O. oleaster* leaves extract against Cd toxicity may be attributed to its antioxidant activities.

Keywords | Cadmium; *Olea oleaster*; Antioxidants; Blood; Rats; Toxicity

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INTRODUCTION

Environmental pollution is a global issue caused by factors like population growth, economic growth, deforestation, agricultural development, urbanization, and industry. Heavy metals are essential for life's development and survival, while trace elements are found in small concentrations in biological systems (Stankovic and Stankovic, 2013). Concern over the declining quality of the environment led to the implementation of a considerable number of experiments on trace elements. In plants, animals, and humans, the elementary constituents are divided into major and trace elements, with the latter category including both essential and non-essential elements; such as toxins (Asati et al., 2016). Heavy metals are dangerously prevalent in the environment and are considered useless to humans, as there is no specific and known mechanism for maintaining their homeostasis (Ding et al., 2021; Vieira et al.,

2011). It is known that heavy metals are highly toxic to living organisms and have harmful effects on human health such as effects on vital organs in the body and cause cancer (ATSDR, 2003; Chou and Harper, 2007; Jomova and Valko, 2011; King et al., 2015). Cadmium (Cd) belongs to the heavy metals and is found in the earth's crust along with other heavy metals such as Cu, Pb, Ni and Zn. Cd is widely used in several industries such as paint, batteries, and alloys. Humans can be exposed to this metal by ingestion and inhalation (Sharma et al., 2015). However, Cd toxicity causes acute and chronic effects in food chains and body tissues, including stomach pain, diarrhea, reproductive, renal, hepatic failure, bone integrity, immune system, nervous system, mental disorders, and cancer. It pollutes soil, surface water, and plants, causing soil organic matter to bind with Cd (Sharma et al., 2015).

Historically, medicinal plants have been one of the most important sources for finding new treatments for human health problems (Sofowora, 1996). History of using medicinal plants is parallel with human's development and understanding of nature. The alternative methods of medicine (as distinct from allopathy) have played an important role in the health care sector all over the world (Al-Attar et al., 2017; Ali et al., 2023). The olive tree (*Olea europaea* and *Olea oleaster*), family: Oleaceae has been used for the treatment of different diseases. Olive trees originate in the eastern Mediterranean and the Kingdom of Saudi Arabia, with compounds like oleuropein, flavonoids, and phenolics (Sofowora, 1996). Animal studies on the influences of olive leaves extracts or their components have revealed several therapeutic effects such as hypoglycemic, anti-tumor and antimicrobial activity (Gürbüz and Öğüt, 2020; Morandi et al., 2021; Sánchez-Gutiérrez et al., 2021). The present study may be the first investigation aimed to evaluate the influence of olive (*Olea oleaster*) leaves extract on male rats exposed to Cd.

MATERIALS AND METHODS

Olive leaves collection and Extraction

Olive leaves were collected from Al Baha region, Saudi Arabia, and carefully dried to obtain superior quality leaves. The leaves were crushed to obtain a powder. 200g of powdered leaves were added to 6L of distilled water and boiled for half an hour. The mixture was filtered by filter paper and the filtrate was evaporated at 40°C. The filtration product was used for the experiment (Lafka et al., 2013).

Experimental design

For the purpose of the current study, 40 Wistar male rats weighing from 110-137g were selected and divided into 4 groups (n=10/group). Experiments were carried out under standard laboratory conditions (temperature: 20±2°C; humidity: 55%±10; 12:12h light: dark cycle). The first group was identified as a control group receiving saline solution orally. The second group was orally exposed to Cd at a concentration equal to 15 mg/kg of body weight per day. The third group was orally treated with olive leaves extract at a concentration of 600 mg/kg of body weight, and after 4h, rats were exposed to Cd as the case in the second group. The fourth group was treated with olive leaves extract as in the third group but was not exposed to Cd. After 6 weeks, blood samples were collected from all groups, and serum samples were obtained by centrifugation process. Blood serum samples were kept an 80°C. Serum analyses were performed to determine the values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, albumin, creatinine, blood urea nitrogen (BUN), uric acid, malondialdehyde (MDA) and catalase (CAT) were measured using *specific* assay kits. Tumor necrosis factor-α (*TNF-*α) and interleukin 6 (IL-6) were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the instructions of manufacturer.

Statistical Analysis

All data are presented as mean ± standard deviation (S.D.). The data was subjected to statistical analysis by conducting analysis of one-way analysis of variation (ANOVA) using SPSS software package (version 22). $P \leq 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the levels of ALT, AST, ALP, total bilirubin, total protein and albumin in all groups. The results of measuring ALT level indicate a significant increase in the second group (P≤0.000) and the third group (P≤0.002) compared to the first one. The level of AST was significantly evoked in the second group (P≤0.000) and the third group (P≤0.01). The results showed that the level of ALP was enhanced in rats exposed to Cd (P≤0.000) and olive leaves extract plus Cd (P≤0.05). Statistical significant increases of total bilirubin were observed in rats of the second group (P≤0.002) and the third group (P≤0.01). The levels of total protein (P≤0.000) and albumin (P≤0.01) were declined in rats of the second group.

Fig. 1A-C shows increased levels of creatinine (P≤0.000), BUN (P≤0.001) and uric acid (P≤0.000) in Cd-exposed rats. In the third group, BUN (P≤0.05) and uric acid (P≤0.05) were increased compared to the control group. Significant increases of MDA (P≤0.001), IL-6 (P≤0.000) and TNF- α (P≤0.000) were noted in rats of the second group (Fig. 2A, 2C and 2D), while statistical significant decreases of CAT (P≤0.000) were detected in rats of the second group (Fig. 2B). Additionally, the results revealed

Table 1: Serum concentrations of ALT, AST, ALP, total protein and albumin in different treatments groups of rats after six weeks. Four groups were assigned for different treatments as; G1: Control (CTL), G2: Cd, G3: Olive leaves' extract plus Cd (OLE+Cd), G4: OLE. All data were expressed as mean ± SD for 10 animals (n=10). Letters (*a, b, ab*) denote significance difference at P<0.05.

Serum Parameters	G1 Control (CTL)	G2 Cd	G3 OLE + Cd	G4 OLE
ALT (U/L)	38.00 ± 2.97 *b*	98.33 ± 11.20 *a*	57.83 ± 9.58 *a, b*	40.67 ± 3.45 *b*
AST (U/L)	100.33 ± 5.20 *b*	207.17 ± 10.48 *a*	134.17 ± 16.69 *a, b*	98.83 ± 6.97 *b*
ALP (U/L)	152.00 ± 6.26 *b*	288.00 ± 16.17 *a*	194.50 ± 16.63 *a, b*	152.33 ± 8.57 *b*
Bilirubin (µmol/L)	0.98 ± 0.18 *b*	2.18 ± 0.44 *a*	1.47 ± 0.24 *a, b*	0.93 ± 0.12 *b*
T. Protein (g/L)	66.00 ± 3.52 *a*	52.83 ± 2.32 *b*	66.00 ± 2.00 *a*	57.17 ± 2.64 *ab*
Albumin (g/L)	35.00 ± 2.90 *ns*	28.50 ± 5.39 *ns*	34.50 ± 2.88 *ns*	36.00 ± 2.76 *ns*

significant increases of MDA (P≤0.02), IL-6 (P≤0.001) and TNF- *α* (P≤0.001) in rats of the third group with decline of CAT (P≤0.04).

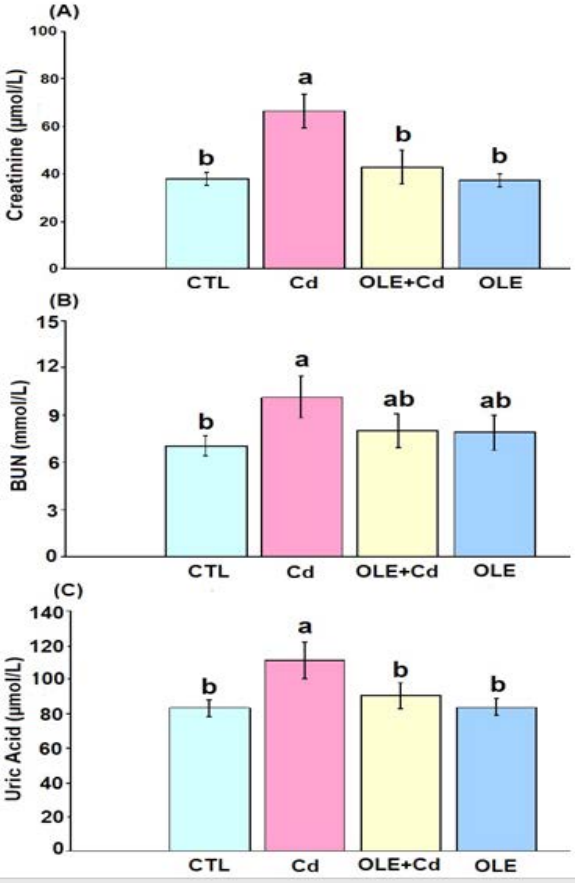

Figure 1: Serum concentrations of creatinine, blood urea nitrogen (BUN) and uric acid were shown in Fig. 1A, B and C, respectively. Four groups were assigned for different treatment throughout 6 weeks as; G1: Control (CTL), G2: Cd, G3: Olive leaves' extract plus Cd (OLE+Cd), G4: OLE. All data were expressed as mean ± SD for 10 animals (n=10). Letters on bars (a, b, ab) denote significance difference at P<0.05.

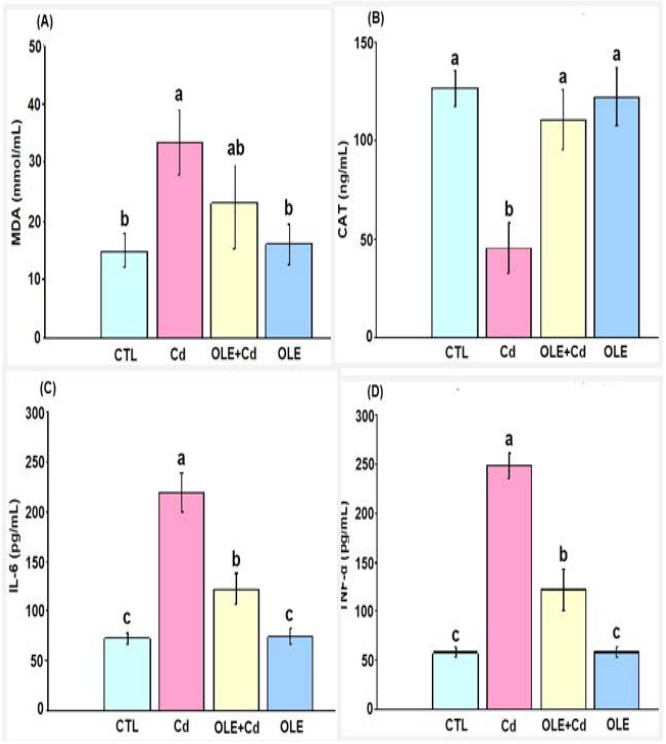

Figure 2 A-D: Serum concentrations of the lipid peroxidation marker; malondialdehyde (MDA), catalase (CAT), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were shown in Fig. 2A, B and C, and D, respectively. Other explanations were given in Fig. 1.

DISCUSSION

Environmental pollution, including Cd, is a major cause of diseases and deaths. This study evaluates the effect of olive leaves extract on rats exposed to Cd. Results show increased serum ALT, AST ALP, and bilirubin levels, while decreasing total protein and albumin, ALT, AST, ALP, bilirubin indicators aid in diagnosing liver function. The blood level of these markers rises when there are abnormalities in liver function. Additionally, the drop in total protein and albumin levels is a sign of liver injury. However, the current outcomes are in line with earlier research

using Cd-exposed experimental animals (Andjelkovic et al., 2019; Poli et al., 2022; Yang et al., 2021).

In this study, kidney function was evaluated by measuring the levels of serum creatinine, BUN and uric acid. Increasing levels of these parameters indicate a defect in kidney function. Similarly, previous studies indicate high levels of these parameters when treating experimental animals with Cd (Iserhienrhien and Okolie, 2022; Poli et al., 2022; Zhao et al., 2022). The present results show that the exposure to Cd induced oxidative stress and inflammation that have been confirmed by the decrease of CAT, and the increase of MDA, *TNF-α* and IL-6 levels. These results are consistent with previous studies (Poli et al., 2022; Zhao et al., 2022). MDA and CAT *are used as indicators of oxidative stress markers. TNF-α* is well known as an inflammatory cytokine. In acute inflammation, macrophages/monocytes produce *TNF-α*, and it is responsible for a various range of intracellular signaling events, causing necrotic or apoptotic processes (Jang et al., 2021). IL-6 is a multifunctional cytokine synthesized in response to stimuli such as inflammation and tissues damage (Ekici et al., 2022; Tanaka et al., 2018).

Currently, there is more interest in medicinal plants and natural products due to their effective treatment properties (Abdel Ghfar et al., 2022; Abdel Ghfar et al., 2023; Greenwell and Rahman, 2015). Many studies indicate the antioxidant capacity of these medicinal plants and natural products with promising therapeutic properties, furthermore they safe, easily available and cheaper in most of the cases (Al Syaad et al., 2023; Hussein et al., 2022). Interestingly, the present results indicate the ability of olive leaves extract to protect against the physiological effects of Cd toxicity in rats, and this is confirmed by indicators of oxidative stress (MDA and CAT), and inflammation and immunopathy markers (*TNF-α* and IL-6). This indicated the effectiveness of olive leaves extract in prevention of Cd toxicity. The possible mechanism of olive leaves extract as a protective factor may be due to its antioxidant properties which impair the activation of Cd into the reactive form. Previous investigations by (Al-Attar et al., 2017) indicated the ability of *O. oleaster* extract to reduce the toxic effect of thioacetamide in mice and Pb in rats through physiological and histpathological evaluations. Oleuropein is a phenolic compound and the main glycoside present in olive leaves and the oil of its fruit. The antioxidant, anti-inflammatory, anti apoptotic and anti-cancer roles of oleuropein were detected by several experimental studies (Asgharzade et al., 2020; Hsu et al., 2021; Lemonakis et al., 2022).

CONCLUSION

Certainly, this study confirms the ability of olive leaves extract to protect against Cd toxicity and reduce its effects, which gives biological indications to encourage the use of olive leaves extract and its active components to treat Cd toxicity and in a way that stimulates the use of this extract to treat the effects of other chemical pollutants and pathological factors.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

NOVELTY STATEMENT

The present study highlights the ability of the O. oleaster leaves' extract could nearly normalize those blood parameters for protection against cadmium toxicity.

AUTHORS CONTRIBUTION

Fatimah A. Al-Saeed: conceptualization, methodology, original draft, investigation, visualization, formal analysis, revision, and editing. Author have read and approved the final manuscript.

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