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Effect of *Phoenix dactylifera* seeds extract on cadmium-induced hepatotoxicity in male mice

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Abstract

Heavy metals are common contaminants that have negative impacts on the body's organs and systems. Date (*Phoenix dactylifera*) has a great interest in biomedical applications and traditional medicine for the management of several diseases. This study evaluated the impact of *Phoenix dactylifera* seed extract (PDSE) on cadmium (Cd)-induced hepatotoxicity in mice. Fifty mice were equally divided into five groups, G1 was negative control, G2 was injected i.p. with PDSE (300 mg/kg b.wt) daily, G3 was injected i.p. with Cd (6.5 mg/kg b.wt) daily, G4 was injected with Cd and PDSE as in G2 and 3, respectively. G5 was injected with Cd as in G3 and then with EDTA (25 mg/kg b.wt). On day 15, sera samples were collected for biochemical parameters assessment. Liver tissues were collected for the determination of oxidants/antioxidants biomarkers and histopathological investigations. The results showed that treatment with PDSE significantly ameliorated the hepatic dysfunctions in the Cd-intoxicated mice evidenced by significant improvement in the ALT, AST, ALP, and total protein levels as well as in the total bilirubin and GGT levels. Furthermore, there was a significant increase in the SOD and CAT activities with a significant decrease in MDA levels after treatment of Cd-injected mice with PDSE. Also, treatment with PDSE resulted in improvement in the hepatic architectures alterations induced by Cd. PDSE showed promising metal chelating activities *in vitro* and in Cd-intoxicated mice by ameliorating biochemical and histopathological alterations in the liver tissues induced by Cd of mice.

Keywords: *Phoenix dactylifera*; Heavy metals; Cadmium; Hepatotoxicity

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Introduction

Due to their prevalence in the environment, heavy metals from various sources have a variety of harmful effects [1, 2]. These substances are naturally occurring trace elements in the environment, but industrial and mining activities have raised their concentrations [3]. Cadmium (Cd), a heavy metal, is ranked among the top 10 most dangerous substances. Due to its lack of essentiality in living organisms, it is poisonous to plants and animals and enters humans through the food chain [4]. The heavy metal Cd is a widespread environmental contaminant that has been linked to significant organ damage in both humans and animals [5]. Acute hepatotoxicity, a well-researched experimental toxicological paradigm, and metal buildup in the liver are the primary effects of acute cadmium exposure in experimental animals [6]. Cd-induced liver damage is thought to be a two-phase process, with the first stage being brought on by direct metal interactions and ischemia, and the second stage being brought on by inflammation and oxidative stress [7, 8]. There is evidence that exposure to Cd causes liver cancer in humans and rodents [9]. It has been observed that various antioxidants and metal-chelating compounds can prevent liver damage caused by Cd [10, 11]. It has been demonstrated that royal jelly may protect against Cd-induced hepatotoxicity in mice by reducing oxidative stress and increasing the expression of Nrf2 [12]. Furthermore, it has been reported that melatonin reduces oxidative stress, inflammation, and apoptosis to protect against Cd-induced hepatotoxicity in mice [13].

Biomedical applications are of tremendous interest to date (*Phoenix dactylifera*). The main byproduct of date fruit, which is produced in enormous quantities, is date seed. These seeds constitute approximately 10% of the fruits, it contains numerous promising biochemical constituents [14, 15]. Due to their rich mineral and antioxidant content, *P. dactylifera* seeds (PDS) have been utilized in conventional medicine to treat liver problems. have been used in traditional medicine for the management of liver diseases due to their high content of minerals and antioxidants [16]. PDS has the potential to be utilized as an ingredient in the pharmaceutical, cosmetic, and food sectors [17]. According to a previous study, PDS is free of any hazardous side effects and is a significant source of phenolic acids, which are hydroxylated derivatives of benzoic acid and cinnamic acid and have antioxidant properties [18]. Free radical scavenging, antioxidant, antimutagenic, antibacterial, anti-inflammatory, gastroprotective, hepato-renal protective, and anticancer effects of the PDS have been demonstrated in preclinical research [19]. This study aims to investigate the potential hepato-protective impact of *Phoenix dactylifera* seeds extract (PDSE) on Cd-induced hepatotoxicity in mice.

Materials and Methods

Chemicals

Cadmium chloride ($CdCl_2$) and Na_2EDTA were purchased from Merck Company (Darmstadt, Germany). All kits were purchased from Bio-Diagnostic Company (Cairo, Egypt).

Preparation of *Phoenix dactylifera* seeds extract

Phoenix dactylifera seeds (PDS) were collected from date fruits purchased from a local market in El-Gharbiah governorate, Egypt, then transferred into the laboratory. The plant materials were identified and authenticated by taxonomists in the Botany Department, Faculty of Science, Tanta University. Seeds were grinded in a mechanical mortar and 50 g of the powder was mixed vigorously with 500 mL 70% (v/v) ethanol. PDSE was filtered, the solvent was air-dried and concentrated in a vacuum evaporator, and then the extracts were weighed, suspended in 0.9% sterile saline, and stored at 20 $^{\circ}$C for further use.

Mice and experimental design

Swiss albino mice (20±2 g) were obtained from the National Research Center (NRC, Cairo, Egypt), then housed under laboratory conditions of temperature and humidity. The experimentation, transportation, and care of the animals were performed and handled in compliance with the ethical guidelines approved by the animal care and use committee, Faculty of Science, Tanta University, Egypt (IACUC-SCI-TU-0264), and according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1996). Fifty mice were divided into five groups (10/each). Group 1 (G1) was used as a negative control. G2 was injected i.p. with PDSE daily (300 mg/kg b.wt) which equaled one-tenth of LD50. G3 was injected i.p. with Cd daily (6.5 mg/kg b.wt) [20]. G4 was injected with Cd and PDSE in the same dose as in G2 and 3, respectively. G5 was injected with Cd as in G3 and then with EDTA as a standard chelating agent (25 mg/kg b.wt). All treatments were continued for 15 consecutive days; at day 15, sera samples were collected for biochemical parameters assessment. Liver tissues were prepared for the determination of oxidants/antioxidants biomarkers and histopathological investigations.

Biochemical analyses

Serum alanine transaminases (ALT) (Cat. no. AL 1031), aspartate transaminases (AST) (Cat. no. AS 1061), alkaline phosphatase (ALP) (Cat. no. AP 1020), total protein (Cat. no. TP 2020), total bilirubin (Cat. no. BR 1111), and GGT (Cat. no. GT 1471) were determined. Hepatic superoxide dismutase (SOD) (Cat. no. SD 2521), catalase (CAT) (Cat. no. CA 2517), and malondialdehyde (MDA) (Cat. no. MD 2529) were estimated according to the manufacturer's protocol.

Histopathological investigation

Liver tissue specimens of liver were harvested and fixed in 10% formalin. Paraffin blocks were prepared after completing the tissue processing in different grades of alcohol and xylene. Sections (5 µm) were prepared from paraffin blocks using a microtome, stained with hematoxylin and eosin, which were observed under a light microscope (Optika, B-350, Ponteranica, Bergamo, Italy) to examine gross cellular damage.

Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the significant differences among treatment groups. The SPSS statistics program was used for data analysis. Dunnett's test was used to compare all groups against the control group to show the significant effect of treatment. The criterion for statistical significance was set at $p \leq 0.05$. All data are presented as mean ±SD.

Results

Treatment with PDSE mitigated the hepatic dysfunctions in the Cd-intoxicated mice

Compared to the negative control group (G1), the Cd-injected group (G3) did show a significant increase ($p \leq 0.05$) in the serum ALT, AST, and ALP activities, with a significant decrease in the total protein levels. However, Cd-injected mice that were treated with PDSE (G5) did show a significant decrease ($p \leq 0.05$) in the previous parameters as compared to the Cd-injected mice alone (Table 1).

Table 1. Serum activities of ALT, AST, and ALP in the different groups under the study

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein (g/dL)
G1	38.0 ± 2.6 [a]	133.2 ± 5.2 [a]	95.45 ± 3.76 [a]	0.72 ± 0.08 [a]
G2	34.0 ± 2.8 [a]	127.5 ± 4.8 [a]	92.57 ± 3.25 [a]	0.74 ± 0.09 [a]
G3	94.7 ± 4.5 [b]	229 ± 7.5 [b]	285.64 ± 5.27 [b]	0.31 ± 0.05 [b]
G4	53.5 ± 2.9 [c]	172 ± 5.5 [c]	169.23 ± 4.76 [c]	0.55 ± 0.07 [c]
G5	74.5 ± 4.6 [d]	195 ± 10 [b,c]	206.87 ± 4.56 [d]	0.45 ± 0.05 [b,c]

The values represented as means ± S.D.; G1: Negative control; G2: PDSE administered; G3: Cd-intoxicated; G4: Cd/PDSE; G5: Cd/EDTA; PDSE: *Phoenix dactylifera* seeds extracts; Cd: Cadmium; EDTA: Ethylene diamine tetra-acetic acid; ALT: Alanine amino transaminase; AST: Aspartate aminotransferase; ALP: Alkaline Phosphatase. This means that do not share a letter are significantly different ($p \leq 0.05$).

Treatment with PDSE ameliorates changes in the total bilirubin and gamma-glutamyl transpeptidase levels

Treatment with Cd (5 mg/kg b.wt) for a month led to a significant increase ($p < 0.01$) in the serum levels of total bilirubin (1.14 ± 0.07 mg/dL), and gamma-glutamyl transpeptidase (GGT) activities (20.31 ± 1.34 U/L) when compared to control groups. Compared to the group of mice that had been treated with Cd alone, treating Cd-injected mice with PDSE led to a significant decrease ($p < 0.05$) in the serum levels of total bilirubin, and GGT, which represented 00.71 ± 0.06 mg/dL, and 14.87 ± 0.95 U/L, respectively. Treatment of Cd-intoxicated mice with EDTA showed improvement in the serum levels of total bilirubin, and GGT but not much as PDSE treatment represented 0.80 ± 0.06 mg/dL, and 17.45 ± 1.45 U/L, respectively (Figure 1).

Impact of the treatment with PDSE on the oxidant/antioxidant hemostasis in Cd-intoxicated mice

Mice that were injected with Cd (G3) showed a significant decrease ($p \leq 0.05$) in the SOD and CAT activities when compared to the negative control group (G1). Furthermore, the Cd-intoxicated mice showed a significant increase ($p \leq 0.05$) in the MDA level when compared to the control group. However, the Cd-injected mice that were treated with PDSE did show a significant increase in the SOD and CAT activities, accompanied by a significant decrease ($p \leq 0.05$) in the MDA level when compared to Cd-injected mice (Table 2).

Treatment with PDSE improved the histopathological alterations in the liver that were induced with Cd injections

Examination of liver sections from the negative control and PDSE control groups showed normal hepatic architectures with no histopathological changes. Normal-like hepatic anatomy, a normal central vein, a normal radiating hepatic strand, normal blood sinusoids, and normal phagocytic Kupffer cells were observed (Figures 2A and B). The examination of the liver sections of the Cd-intoxicated mice showed loss of cellular organization, deteriorated cells changed lobular shape, nuclear degradation, and disarrangement of normal hepatic cells. Congestion of the hepatic portal vein was detected. Some hepatic cells showed degenerated alteration (pyknotic nuclei) (Figure 2C). The liver sections of the Cd-injected mice that were treated with PDSE revealed normal-appearing hepatic anatomy, a normal central vein, and a normal radiating hepatic strand, with normal blood sinusoids and phagocytic Kupffer cells, certain hepatic cells displayed minor degree of degeneration with no congestion and pyknotic nuclei were seen (Figure 2D). The liver sections of the Cd-intoxicated mice that were treated with EDTA showed moderate hepatic central vein congestion with a mild degree of degenerated hepatocytes and widening blood sinusoids (Figure 2E).

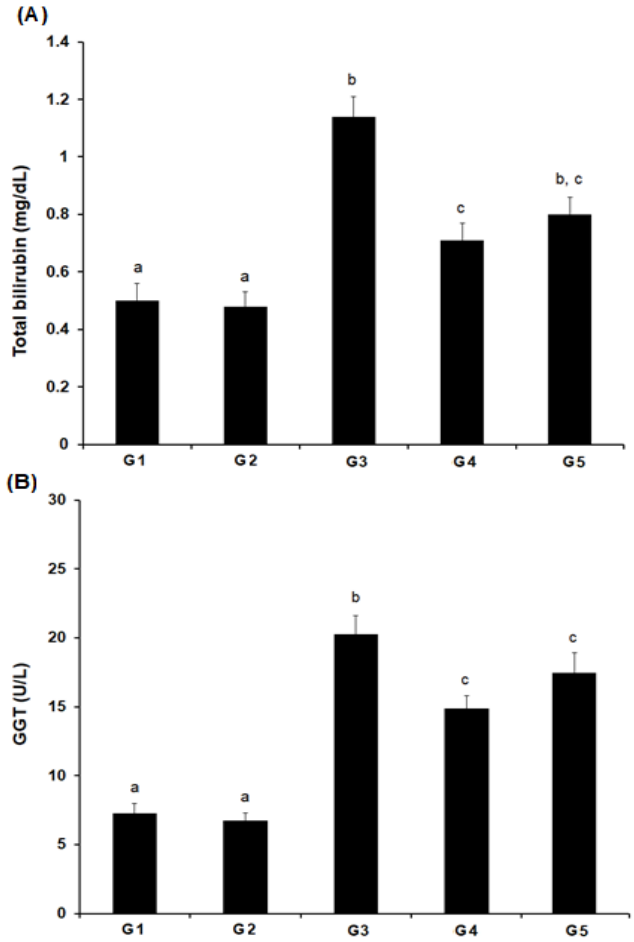

Figure 1. Serum total bilirubin levels (A), and gamma-glutamyl peptidase (GGT) **(B)** in the different groups under the study. The values represented as means ± S.D.; G1: Negative control; G2: Cd-intoxicated (5 mg/kg b.wt); G3: Cd/PDSE; G4: EDTA-injected (25 mg/kg b.wt); G5: Cd/EDTA. This means that do not share a letter are significantly different ($p < 0.05$).

Table 2. Hepatic SOD, CAT, and MDA levels in the different groups under the study

Groups	SOD (U/g tissue)	CAT (µM/min/mg tissue)	MDA (nmol/g tissue)
G1	3.87 ± 0.15 [a]	78.45 ± 3.7 [a]	35.33 ± 1.9 [a]
G2	4.05 ± 0.23 [a]	84.25 ± 2.9 [a]	30.31 ± 1.5 [a]
G3	1.65 ± 0.09 [b]	47.67 ± 2.5 [b]	70.57 ± 2.5 [b]
G4	2.90 ± 0.13 [c]	62.76 ± 2.8 [c]	49.52 ± 2.3 [c]
G5	2.04 ± 0.14 [b,c]	51.98 ± 2.3 [c]	55.76 ± 2.4 [d]

The values represented as means ± S.D.; G1: Negative control; G2: PDSE administered; G3: Cd-intoxicated; G4: Cd/PDSE; G5: Cd/EDTA; PDSE: *Phoenix dactylifera* seeds extracts; Cd: Cadmium; EDTA: Ethylene diamine tetra-acetic acid; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde. This means that do not share a letter are significantly different ($p < 0.05$).

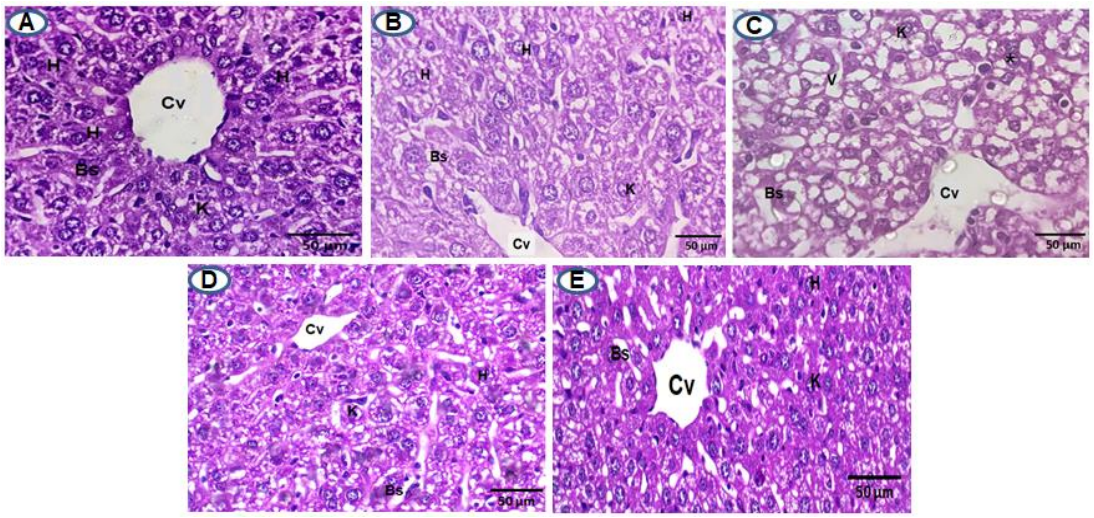

Figure 2. Photomicrographic liver section of the negative control group (A), PDSE control group (B), Cd-intoxicated group (C), Cd/PDSE treated group (D), and Cd/EDTA treated group (E).

Discussion

The heavy metal Cd is a very toxic metal that can cause cancer. It is dispersed throughout the body by way of blood flow, but accumulates and becomes poisonous in the liver, kidney, reproductive system, and lungs [21, 22]. These harmful effects of Cd may result from oxidative damage, genotoxic mechanisms, the induction of a complex network of inflammatory mediators, interference with metabolism, and loss of vital trace elements [22, 23]. When cadmium enters the body, it reacts with metallothionein and accumulates in both soft tissue and solid organs, particularly the liver, which is the principal organ impacted by exposure to toxic substances [24]. One of the abundant sources of polyphenols and flavonoids is the seed of *Phoenix dactylifera* [25]. Its pharmacological effects, such as antioxidants, anti-inflammatory, anti-cholesterol, and antidiabetic, have been thoroughly studied [25-28].

The therapeutic effect of PDS against Cd toxicity is not investigated. Therefore, the current study evaluated the potential therapeutic effects of PDSE against Cd-induced hepatotoxicity in mice. The release of intracellular enzymes like ALT and AST into circulation is one of the most significant effects of hepatocyte damage caused by Cd treatment. The increased activity of these enzymes is a sign that the cell membranes' functional integrity has been compromised and that the liver is leaking enzymes [29, 30]. The results showed that injecting rats with Cd (6.5 mg/kg b.wt) led to a significant increase in serum levels of ALT, AST, and ALP with a significant decrease in total protein levels. These findings agreed with previous studies that reported the hepatotoxic effect of Cd which is evidenced by high serum levels of liver enzymes [12, 13]. Treatment of Cd-injected mice with PDSE led to a significant decrease in the previous parameters in serum, this is in accordance with the present study which demonstrated that treatment with natural products effectively protected against hepatotoxicity caused by cadmium exposure in experimental animals [31, 32].

According to prior research, Cd-induced inflammation and oxidative stress lead to liver tissue

damage due to hepatocellular necrosis and apoptosis [21, 34]. Additionally, the activation of inflammatory cells results in the production of reactive oxygen species (ROS) and lipid peroxidation, which is linked to cadmium hepatotoxicity as a crucial event [35, 36]. The current study demonstrated that the Cd-intoxicated mice showed a significant decrease in the SOD and CAT activities, accompanied by a significant increase in the MDA level when compared to the control group. However, the Cd-injected mice that were treated with PDSE did show a significant increase in the SOD and CAT activities, and a significant decrease in the MDA level when compared to Cd-injected mice. These findings agreed with previous studies that reported the hepatic oxidative stress in Cd toxicity and the role of natural products in enhancing antioxidants/oxidants hemostasis [31-33]. Furthermore, the results revealed a significant decrease in bilirubin and GGT levels in mice treated with PDSE after Cd intoxication. This could be due to the potential hepatoprotective effects of PDSE on liver tissues26. This was in line with previous studies that reported the impact of PDSE in experimental animals [26, 33].

The Cd-intoxicated mice showed loss of hepatocellular organization and congestion of the hepatic portal vein. A previous study reported the negative effect of Cd injection on the histological architecture of hepatocytes [30]. Treatment with PDSE after Cd intoxication improved the histopathological alterations in the liver tissues that were induced by Cd injection. The liver sections of the Cd-injected mice that were treated with PDSE revealed normal-appearing hepatic cells displayed a minor degree of degeneration with no congestion. These findings were in accordance with the previous report [36].

Conclusion

This study highlighted data indicating PDSE may protect mice's livers from damage brought on by Cd. PDSE improved the biochemical and histological alterations in the liver tissues brought on by the mice's Cd exposure.

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