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Caffeic acid phenyl ester prevents cadmium intoxication induced disturbances in erythrocyte indices and blood coagulability, hepatorenal dysfunction and oxidative stress in rats

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ABSTRACT

Here we investigated the protective role of caffeic acid phenyl ester (CAPE) on erythrocyte indices and osmotic resistance, blood coagulation, hepato-renal function and antioxidant status in cadmium (Cd) toxicity in rats. Cd intoxication was induced by intraperitoneal injection (i.p.) of cadmium chloride (1 mg/kg/day) for 21 days, and CAPE was daily given (10 μmol/kg; i.p.) also for 21 days. At day 22, blood samples, livers and kidneys were prepared for screening of: (1) erythrocyte indices: red blood cell (RBC) count, osmotic fragility, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC); (2) blood coagulation tests: prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB) level; (3) serum levels of liver and kidney function biomarkers (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, creatinine and blood urea nitrogen); (4) blood, liver and kidney levels of Cd; and (5) serum and hepato-renal concentrations of glutathione (GSH), superoxide dismutase (SOD), and thiobarbituric acid reactive substances (TBARS). Cd intoxication significantly impaired hepato-renal function, prolonged PT and APTT, reduced FIB, decreased RBC count and osmoresistnacy as well as the values of HGB, HCT, MCV, MCH and MCHC. Interestingly, therapy with CAPE successfully eliminated Cd and significantly stabilized erythrocyte indices, blood coagulability and hepato-renal functional status in Cd-intoxication. Additionally, CAPE therapy significantly reversed the decreases in GSH and SOD, and the increases in TBARs that were induced by Cd intoxication. In conclusion, CAPE can represent a promising therapeutic agent in eliminating Cd and counteracting its hematological, hemostasis and hepatorenal toxic effects.

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Introduction

Caffeic acid phenyl ester (CAPE) is one of the main medicinal components of honeybee propolis that possesses a variety of biological and pharmacological actions such as potent free radical scavenging, antioxidant, anti-inflammatory, cytoprotective, immunomodulatory, antiviral and promising anticancer properties [1]. Recently, the ameliorating effects of CAPE on different disease modalities of hematological, blood coagulation and vascular abnormalities have also been emerged. In this concept, CAPE had shown to ameliorate blood coagulation abnormalities and disturbed oxidative stress in endotoxic model of acute liver failure [2], increase cerebral blood flow and improve ischemic stroke in neurovascular disease [3], protect peripheral blood mononuclear cells against hyperthermal stress [4], prevent drugs to induce toxic and damage effects on red blood cells (RBCs) [5], and potently inhibit the synthesis of inflammatory and atherosclerotic leukotrienes in human polymorphonuclear leukocytes and whole blood [6].

Cadmium (Cd) is classified as a very harmful environmental pollutant to the humans that transfers between various levels of the food chain [7]. Occupational exposure to the Cd and its compounds primarily occurs in mining, smelting, processing, and battery manufacturing. In addition, environmental and non-occupational exposures come from various foods, contaminated water, contaminated dust and tobacco smoke [8]. Though the definite mechanisms of its associated toxicity are not yet well covered, it has been revealed that Cd markedly stimulates the formation of reactive oxygen species (ROS), enhances lipid peroxidation, cell membrane damage, and depletes the antioxidant defense elements in different body organs [9]. It has been proved that after exposure, Cd enters the blood and binds to the erythrocyte membranes and blood albumin, and then is transported to liver, where it bounds to metallothionein (MT) [10]. The Cd–MT complex is then released back into circulation [10], and accumulates in the blood system, kidney, liver, lung, testis, brain, and bone [11]. In the blood and tissues, Cd stimulates the formation of ROS, thus causing oxidative damage, which result in a loss of cell membrane functions [12], multi-organ damage and important hematological alterations [13, 14].

Over the past decade, a variety of research studies have reported that medications with free-radical scavengers and antioxidants are useful in protecting against Cd toxicity [7, 14, 15]. To date, few studies have shown the remarkable tissue protective effects of CAPE against Cd intoxication. In this regard, therapy with CAPE had significantly resulted not only in elimination of Cd from blood and tissues but also in preventing Cd-induced oxidative stress, overproduction of ROS, impaired cellular ultrastructures, and injuries in the renal, cardiac and liver tissues [9, 16–18]. However, the possible preventative effect of CAPE against the hematological and blood coagulation dysfunctions secondary to Cd intoxication is still not well investigated. Coherently, the present study aimed to investigate the possible alleviating effects of CAPE on the altered hematological, erythrocyte indices and coagulopathy state as well as the oxidative stress response that could be associated with Cd intoxication in rats.

Materials and methods

Chemicals and reagents

Cadmium chloride ($CdCl_2$) and caffeic acid phenethyl ester (CAPE) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Commercial assay kits of total reduced glutathione (GSH) content, superoxide dismutase (SOD) activity, and thiobarbituric acid reactive substances (TBARS) concentration were purchased from Cayman Chemical (Ann Arbor, MI, USA). All other used chemicals and reagents were of analytical grade and obtained from standard commercial supplies as stated under the sections of their applications.

Animals, treatments and experimental approach

Forty adult male Wistar albino rats, weighing 230–250 g, were used in the present study. The rats were housed five per cage under controlled temperature (20–25 °C) and 12-h light–dark cycle, and allowed free access to water and a commercial rat pellets stock diet. All experimental protocols were approved by the Committee for the Care and Use of Laboratory Animals at Umm Al-Qura University, KSA, and all animals received care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health. The rats were randomly divided into 3 experimental groups: control group ($n = 10$), Cd-group ($n = 15$), and Cd plus CAPE group ($n = 15$). In Cd and Cd + CAPE groups, $CdCl_2$, dissolved in physiological saline (0.9% sodium chloride (NaCl) in distilled water), was intraperitoneally injected at a dose of 1 mg/kg/day for 21 days, and in Cd + CAPE group, CAPE was co-administered i.p. at a dose of 10 μmol/kg for also 21 days. The doses of both Cd and CAPE were chosen on the basic of previous studies [2, 9, 16]. Control rats were received only with physiological saline. At the end of the study (i.e. at day 22), all animal groups were fasted for 12 h and then sacrificed under ether anesthesia and their blood specimens were collected. After blood withdrawal, the livers and kidneys were harvested quickly, and divided into two portions: a portion was weighed and quickly stored at −80 °C until Cd measurement, while the second one was homogenized in RIPA lysis buffer (1:6 w:v), centrifuged at 10 000 rpm for 10 min at 4 °C, and its supernatant was stored at −80 °C until used for measurement the intra-hepatic and intra-renal concentrations of antioxidant and oxidative stress biomarkers as described below.

Blood sample analysis

During scarification process, four blood samples were immediately withdrawn from the vena cava of each rat and used for blood coagulation, hematology and biochemical analyses.

The first sample was collected on a tube contained 0.11 M sodium citrate anticoagulant (1:9, v:v), and used for plasma preparation for screening of the following blood coagulation tests: prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB) concentrations, by using Dade® Behring reagents and following manufacturer's instructions as previously described [19].

The second sample was collected in a tube contained disodium salt of ethylene diamine tetra acetic acid (EDTA) anticoagulant and used for determination of the following hematology parameters: counts of RBCs, white blood cells (WBCs) and platelets (PLTs), hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). These hematological parameters were determined by standard hematological techniques.

The third sample was collected into a heparinized test tube and used for determination of erythrocyte osmotic fragility according to the method described previously by Azeez et al. [20] and Mineo et al. [21]. Briefly, 0.02 ml of blood of each rat was transferred into microtubes containing one ml of increasing concentration of phosphate-buffered NaCl solution (0.0, 0.1, 0.3, 0.5, 0.7, and 0.9%) at pH 7.4. The microtubes were then gently mixed, incubated at room temperature for 30 min, and then centrifuged at 2000 rpm for 15 min. The supernatant (200 μl) of each microtube was decanted into 96-well micro-plate, and its optical density was determined spectrophotometrically at 540 nm (Microplate reader, Bio-Rad Laboratories). The percentage of RBC hemolysis in each NaCl concentration was determined, using hemolysis in distilled water (0.0% NaCl) as the maximum percentage. The higher hemolysis occurs, the greater is osmotic fragility of RBC [20, 21].

The last portion of the collected blood was placed in a plain centrifuge tube without any anticoagulant and after centrifugation process, its corresponding serum was obtained and used for measurement the sera concentrations of Cd and biomarkers of antioxidation and oxidative stress as described below.

Biochemical analysis of hepato-renal function

The prepared sera samples were employed for measurement of the serum concentrations of liver function enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)), albumin (ALB), and kidney function biomarkers (serum creatinine (CRE) and blood urea nitrogen (BUN)).

Evaluation of antioxidant and oxidative stress status

The levels of total reduced GSH and activities of SOD (as indices of non-enzymatic and enzymatic antioxidant status, respectively) were measured in the serum and homogenates of the liver and kidney tissues. On the other hand, the concentrations of TBARS, indices of lipid peroxidation and oxidative stress, were also determined in these sera, liver and renal biological samples. During these three assays, specific commercial kits (Cayman Chemical; Ann Arbor, MI, USA) were used, and all samples were processed in duplicate and according the manufacturer's instructions.

Measurement of blood, hepatic and renal cadmium content

Cd levels in the blood, liver, and kidneys were determined as described in previous studies [22, 23]. For assessment of Cd levels in blood, 0.5 ml whole blood samples were used, while for its assessment in tissues, constant weight slices of livers and kidneys were oven dried at 60 °C. The blood samples and the dried tissues (100 mg from each sample) were digested with 3 ml trace pure concentric nitric acid on a hot plate at 120 °C. Once the digestion was complete, the samples were cooled at room temperature and their volumes were then adjusted to 10 ml with deionized water. Cd concentration was quantified by using an atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 100). Cd concentration is expressed as μg/ml blood and μg/g liver or kidney tissue weight.

Table I – Hematological and blood coagulation findings

Parameter	Units	Control group ($n = 10$)	Cd group ($n = 15$)	Cd + CAPE group ($n = 15$)
WBC	$10^3/\mu l$	11.92 ± 0.79	13.53 ± 2.72	12.27 ± 1.46
RBC	$10^6/\mu l$	8.67 ± 0.48	$5.93 \pm 0.83^{*}$	$8.10 \pm 0.16^{\#}$
HGB	g/l	157.67 ± 7.23	$115.46 \pm 6.03^{*}$	$151.67 \pm 3.51^{\#}$
HCT	%	48.33 ± 0.71	$41.83 \pm 1.00^{*}$	$46.13 \pm 1.01^{\#}$
MCV	fL	64.27 ± 0.84	$52.87 \pm 4.48^{*}$	$60.20 \pm 1.54^{\#}$
MCH	pg	22.13 ± 1.65	$16.70 \pm 1.11^{*}$	$20.40 \pm 1.20^{\#}$
MCHC	g/l	344.42 ± 39.50	$300.26 \pm 27.77^{*}$	$337.67 \pm 38.50^{\#}$
PLT	$10^3/\mu l$	1000.27 ± 91.83	$775.32 \pm 59.61^{*}$	$933.36 \pm 70.76^{\#}$
PT	Second	12.82 ± 1.37	$43.14 \pm 4.33^{*}$	$17.76 \pm 2.33^{\#}$
APTT	Second	21.73 ± 3.46	$77.52 \pm 13.43^{*}$	$29.36 \pm 5.21^{\#}$
FIB	mg/dl	263.90 ± 33.77	$93.37 \pm 9.37^{*}$	$226.68 \pm 42.23^{\#}$

The values are presented as means ± SD. (Cd) cadmium, (CAPE) caffeic acid phenyl ester, (WBC) white blood cell, (RBC) red blood cell, (HGB) hemoglobin, (HCT) hematocrit, (MCV) mean corpuscular volume, (MCH) mean corpuscular hemoglobin, (MCHC) mean corpuscular hemoglobin concentration, (PLT) platelet, (PT) prothrombin time, (APTT) activated partial thromboplastin time, and (FIB) fibrinogen.

* $P < 0.05$ versus control group.

# $P < .05$ versus Cd group.

Table II – Erythrocyte osmotic fragility

Group	Hemolysis of RBC (%)					
	0.0% NaCl	0.1% NaCl	0.3% NaCl	0.5% NaCl	0.7% NaCl	0.9% NaCl
Control ($n = 10$)	87.7 ± 3.2	80.5 ± 4.5	65.5 ± 7.1	33.5 ± 5.6	13.3 ± 3.3	2.1 ± 0.3
Cd ($n = 15$)	97.3 ± 2.2*	93.3 ± 4.3*	85.3 ± 5.2*	55.7 ± 7.4*	38.4 ± 3.5*	21.5 ± 1.3*
Cd + CAPE ($n = 15$)	89.3 ± 7.4#	84.5 ± 3.6#	68.5 ± 2.7#	37.5 ± 2.1#	17.7 ± 2.2#	3.5 ± 0.7#

(Cd) cadmium, (CAPE) caffeic acid phenyl ester.
* $P < 0.05$ versus control group.
# $P < 0.05$ versus Cd group.

Statistical analysis

The results were expressed as the mean ± standard deviation (SD) and statistical analysis was carried out using SPSS software, version 16.0 (SPSS Inc., Chicago, IL, USA). Differences among the groups were investigated using one-way analysis of variance (ANOVA) followed by a Student's t-test. Differences between percentages of RBC hemolysis for determination of osmotic fragility for the groups were analyzed by χ^2 test. A P value of <0.05 was considered statistically significant.

Results

Hematological and blood coagulation findings

The hematological and blood coagulation changes are shown in Table I. Compared with the control group, Cd-group had showed significant decreases in RBC and PLT counts, but not in WBC counts. Moreover, the values of HGB, HCT, MCV, MCH, and MCHC had significantly decreased in Cd-group in comparison with their values in the normal controls. In contrary, therapy with CAPE had successfully ameliorated these hematological toxic effects of Cd, whereby the counts of RBCs and PLTs, as well as the values of HGB, HCT, MCV, MCH, and MCHC had showed no significant differences than those of normal control rats. Intoxication with Cd had also resulted in significant deteriorations on blood coagulation. As demonstrated in Table I, there were significant prolongation of PT and APTT clotting tests associated with significantly decreased FIB levels in Cd-group, when compared with control group. By contrast, treatment of these Cd-injected rats with CAPE had significantly succeeded in improvement of the values of PT APTT, and FIB (Table I). Collectively, this part of the results indicates that Cd intoxication in rats is associated with important alterations in their hematological and blood coagulation parameters and these alterations were remarkably alleviated by CAPE therapy.

Effects on erythrocyte osmotic fragility

Changes in RBCs hemolytic pattern after their exposed to different concentrations of NaCl solution were measured to determine the erythrocyte osmotic fragility of all animal groups. As shown in Table II, the osmotic fragility of RBCs of Cd group was significantly higher than that of Cd + CAPE and normal control groups at each tested NaCl solution. This in turn reflects the stabilizing effect of CAPE therapy on RBC osmotic resistance.

Biochemical findings

The results of serum biochemistry reflect that both the hepatic and renal function of rats had been altered due to Cd intoxication and these damaging effects of Cd had been attenuated by CAPE therapy. As shown in Table III compared with the control group, significant changes in the serum levels of AST, ALT, ALP, and ALB (as biomarkers of liver function), as well as the serum levels of CRE and BUN (as biomarkers of renal function) were detected only in Cd group but not in Cd + CAPE group.

Blood, renal and hepatic levels of cadmium

Next, we confirmed the accumulation of administered Cd in the blood and hepato-renal tissues of the rats (Fig. 1). After 21

Table III – Serum levels of liver and kidney function biomarkers

Parameter	Units	Control group ($n = 10$)	Cd group ($n = 15$)	Cd + CAPE group ($n = 15$)
AST	IU/l	109.00 ± 12.17	571.33 ± 72.29*	163.00 ± 14.93#
ALT	IU/l	49.17 ± 3.27	136.07 ± 21.06*	73.33 ± 11.35#
ALP	IU/l	206.33 ± 20.79	274.33 ± 25.14*	211.33 ± 17.35#
ALB	g/dl	4.33 ± 0.59	2.86 ± 0.38*	4.01 ± 0.27#
CRE	mg/dl	0.26 ± 0.04	0.41 ± 0.34*	0.29 ± 0.01#
BUN	mg/dl	47.36 ± 9.12	63.65 ± 13.44*	49.22 ± 10.10#

The values are presented as means ± SD. (Cd) cadmium, (CAPE) caffeic acid phenyl ester, (AST) aspartate aminotransferase, (ALT) alanine aminotransferase, (ALP) alkaline phosphatase (ALP), (ALB) albumin, (CRE) creatinine, and (BUN) blood urea nitrogen.
* $P < 0.05$ versus control group.
# $P < 0.05$ versus Cd group.

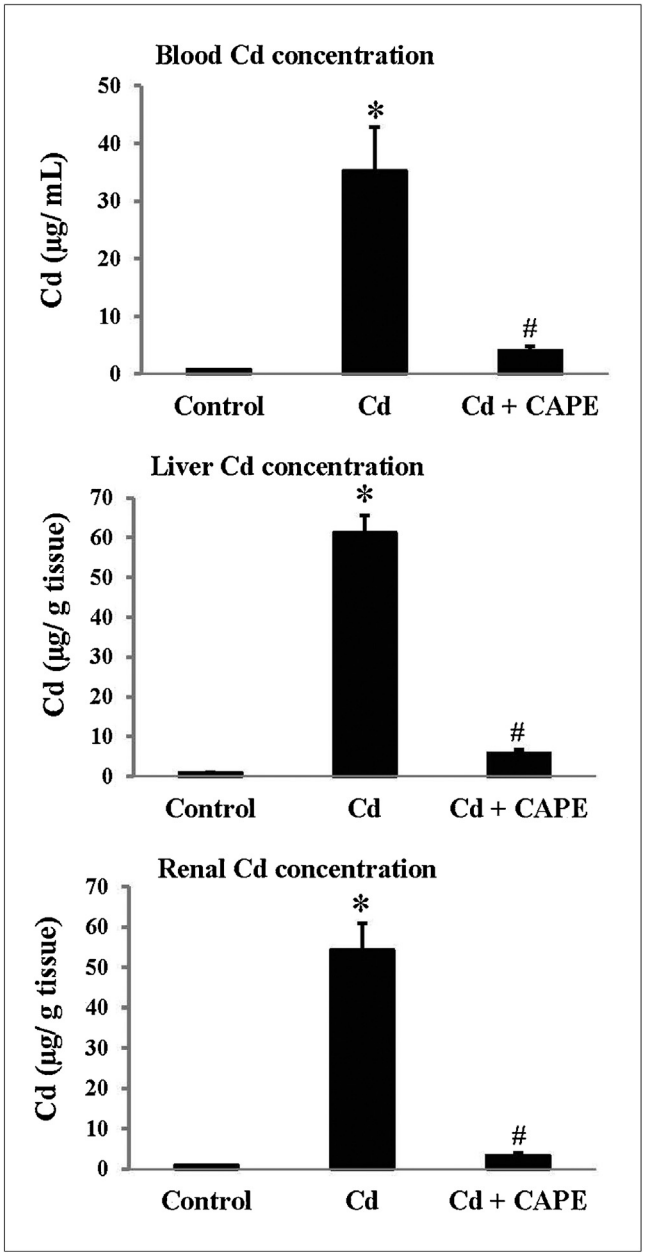

Fig. 1 – Effect of caffeic acid phenyl ester (CAPE) therapy on cadmium (Cd) levels in blood and hepato-renal tissues after 21 days of treatment. *P < 0.001 versus control group; #P < 0.01 versus Cd group

days, Cd levels were very high in the blood, liver and kidney of Cd group. On the contrary, concurrent administration of CAPE significantly eliminated Cd from these biological tissues.

Serum, renal and hepatic levels of GSH, SOD and TBARS

Finally, we measured the levels of GSH (an example of non-enzymatic antioxidant defense mechanism), activities of SOD (an example of enzymatic antioxidant defense mechanism), and concentrations of TBARS (an index of lipid perioxidation and oxidative stress) in the sera samples and the liver and kidney tissue homogenates of all animal groups. As demonstrated in Table IV, Cd intoxication in rats had associated with significant reduction in GSH content and SOD activity, as well as marked elevation in TBARS content in the sera, livers and kidneys of Cd group. On the other hand, concurrent administration of CAPE with Cd had obviously counteracted these effects of Cd on GSH, SOD, and TBARS in all tested biological samples.

Discussion

Cd is classified as a major industrial and environmental pollutant that raises serious public health concerns worldwide because of its high toxic effects on human and animals [8]. CAPE is a biological active component of honeybee propolis extracts with pluripotent pharmacological actions and potent antioxidant activity [9]. This study was designed to investigate the effect of CAPE supplementation therapy on erythrocyte indices, blood coagulation, hepato-renal functional and antioxidant status in Cd intoxication in rats. The results showed that CAPE therapy not only eliminated the heavy metal but also and importantly resulted in an obvious protection against Cd-induced marked hematological and blood hemostasis disturbances, hepato-renal injury and oxidative stress in rats.

There is a compelling body of evidence that Cd exposure importantly contributes to human and animal liver and kidney diseases [24, 25]. After the intake and resorption, Cd enters the blood and binds to the erythrocyte membranes and plasma albumin [26]. In the blood and tissues, Cd stimulates lipid peroxidation and the formation of ROS, thus causing oxidative cellular and tissue damages [10, 12, 13]. In support, Cd was detected at high concentrations in the blood, liver and kidneys of Cd group (Fig. 1), and this accumulation pattern of Cd was significantly attenuated with CAPE therapy. Moreover, the biochemical findings (Table III) revealed the occurrence of hepato-renal impairment in Cd-exposed animals

Table IV – Antioxidant and lipid peroxidation status in serum and hepato-renal tissues

Group	GSH (µmol/mg protein)			SOD (U/mg protein)			TBARS (nmol/mg protein)		
	Serum	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney
Control ($n = 10$)	13.7 ± 3.1	69.9 ± 7.3	57.7 ± 9.2	3.8 ± 0.6	289.5 ± 37.3	267.7 ± 23.4	27.4 ± 4.2	48.6 ± 6.2	37.4 ± 5.9
Cd ($n = 15$)	4.7 ± 0.9*	11.7 ± 1.2*	13.74 ± 1.7*	1.2 ± 0.2*	64.8 ± 8.2*	71.5 ± 6.7*	116.2 ± 23.4*	1019.5 ± 195.9*	1113.3 ± 195.9*
Cd + CAPE ($n = 15$)	12.2 ± 1.2#	75.9 ± 11.4#	68.7 ± 10.2#	3.3 ± 0.8#	305.3 ± 50.5#	311.5 ± 47.8#	36.74 ± 5.8#	87.2 ± 14.6#	118.6 ± 23.29#

The values are presented as means ± SD. (Cd) cadmium, (CAPE) caffeic acid phenyl ester, (GSH) total glutathione, (SOD) superoxide dismutase, and (TBARS) thiobarbituric acid reactive substances.
* $P < 0.05$ versus control group.
\# $P < 0.05$ versus Cd group.

but not in Cd + CAPE-treated animals, as reflected by significant elevations in the serum levels of AST, ALT, ALP, CRE and BUN [27]. The observed hepato-renal protective effects of CAPE against Cd were also accompanied with preventing Cd to induce lipid peroxidation and decrease antioxidant defense system (Table IV). Taken together, these findings can reinforce those previously reported that CAPE has a potent protective effect against renal, liver, and other body organ injuries caused by Cd intoxication, and this might be by eliminating Cd and inhibiting Cd-induced oxidative stress and tissue damage [9, 16–18].

Hematopoietic system is one of the most sensitive systems to evaluate the hazards effects of poisons and drugs in humans and animals [27]. In consistency, the current study indicated that an exposure to toxic Cd was associated with significant disturbances in erythrocyte indices; and therapy with CAPE had successfully alleviated these hematological changes induced by Cd (Table I). In this regard, anemia was clearly observed in rats received Cd alone but not in those received Cd plus CAPE. In agreement, it has been approved that Cd accumulation in kidney, liver and spleen can suppress the activity of these important hematopoietic tissues [28]. Additionally, some previous studies revealed that exposure to Cd induces anemia associated with decrease in RBCs counts, HGB concentration, HCT value, and induction of oxidative damage and lipid peroxidation in blood and RBCs [13, 14, 29, 30]. Moreover, the decreases that were observed her in the values of MCV, MCH, and MCHC in Cd group but not in Cd + CAPE group can also indicate the further ability of Cd intoxication to induce microcytic hypochromic anemia [25], and this effect was also alleviated by CAPE therapy. Furthermore, data of erythrocyte osmotic fragility test (Table II); which is a test refers to the propensity of erythrocytes to hemolyse when they are subjected to osmotic stress by being placed in a hypotonic solution [20, 21], demonstrated that exposure to Cd had resulted in a significant increase in RBC osmofragility and this effect was significantly improved by CAPE treatment (Table II). Recent reports revealed that the enhanced oxidative stress and decreased antioxidant status result in increased erythrocyte deformability, easier RBC membrane lipoperoxidability and damage, and consequently, an increased osmotic fragility and shortened life-span [20, 21]. As the powerful free radical scavenger and antioxidant properties of CAPE have been reported in the present study and by previous researchers [2, 31, 32], it can be considered that a portion of the stabilizing effect of CAPE therapy on rat's RBC osmotic resistance could be attributed to its antioxidant activity. Collectively, Cd intoxication might lead to anemia as a result of either suppression the activity of hematopoietic tissues, impaired erythropoiesis, accelerated erythroclasia because of the altered RBCs membrane permeability, increased RBCs mechanical fragility, and/or defective Fe metabolism [25].

Data of blood clotting tests (Table I) demonstrated that exposure to Cd had resulted in a significant hypocoagulation state in form of marked prolongation of the coagulation tests PT, APTT, low PT count and decreased FIB levels-And, this Cd's hemostatic dysregulation effect was significantly improved by CAPE treatment. It is well known that the liver is the major organ for synthesis of procoagualtion factors and substances. Thus, the hypocoagulation state that was observed her in Cd group but not in Cd + CAPE group might be related to Cd-induced liver injury with decreased production of the procoagulation factors and reduced hepatic clearance of plasminogen activators leaded to enhanced fibrinolytic activity [2, 33]. These findings and its related suggestion can also be supported by Korish's report [2] that therapy with CAFÉ protects the liver and prevents the hemostatic alterations in endotoxic-induced acute liver failure.

Earlier studies had indicated that treatment with free-radical scavengers and antioxidants are useful in protecting against Cd toxicity [7, 14, 15]. Therefore, depletion of antioxidative defense mechanism (represented by decreased SOD and GSH) together with increased TBARs (an index of lipid peroxidation and oxidative stress) that were observed here in the serum, liver and kidney tissues of Cd-injected rats but not of Cd-injected/CAPE-treated rats (Table IV) could be the main underlying pathogenic mechanisms by which the injected Cd had induced its hematological and organ toxicity [9, 14]. Similarly, the preventative effects of CAPE that were also observed against Cd could also be attributed to its potent antioxidant property [2, 32]. In harmony, Gokalp et al. [5] showed the cytoprotective ability of CAPE in preventing anti-TB drug ''isoniazed'' to induce oxidative damage in RBCs, and the potent renoprotective effect of CAPE against Cd-induced injury has been previously confirmed and attributed mainly to the antioxidant activity of CAPE [9, 16–18]. Finally, by increasing the antioxidant elements and inhibiting the oxidative status, CAPE had shown to protect the brain vasculature from ischemic stroke disease [3], and protect the liver and improve blood coagulation abnormalities in endotoxic model of acute liver failure [2].

Conclusion

Based on the presented results, it can be concluded that Cd intoxication was resulted in impaired erythrocyte indices, erythrocyte osmotic resistance blood coagulability, and hepato-renal function in rats, and this might be due to enhancing of lipid peroxide concentration and/or depletion of the activity of antioxidant defense elements. By contrast, CAPE supplementation therapy was resulted in favorable effects in eliminating Cd and preventing the hematological, blood coagulation, and hepato-renal disturbances that occurred secondary to Cd intoxication. Moreover, CAPE therapy was also resulted in restoring the antioxidant activity and inhibiting lipid peroxidation in both blood and organs. Therefore, CAPE could be a promising agent for the treatment of Cd intoxication; however, further studies are crucially needed to improve this treatment in patients.

Authors' contributions/Wkład autorów

TH Ashour and AG El-Shemi – manuscript authors, AG El-Shemi – for correspondence.

Conflict of interest/Konflikt interesu

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Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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