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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.

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