Health Services Vocational Collage

Olive leaf extract (Olea europaea L.) Restores Liver Functions From Cadmium Induced Liver Injury

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Abstract

Cadmium (Cd) is a toxicant metal derived from horticultural and industrial sources. In recent work, we reported that olive leaf extract (Olea europaea L.) (OLE) restores liver functions from cadmium induced liver injury. Rats were pretreated with OLE and consecutively injected with CdCl2 (6.5 mg/kg) for 5 days. To evaluate liver function, alanine aminotransferase (ALT), aspartate aminotrans-ferase (AST) and γ-glutamyl transferase (GGT) were detected in the serum, total antioxidant (TAS) and total oxidative stress (TOS) statuses were established in the liver and expression of caspase 3 were determined for the antioxidative and anti-apoptotic effects of OLE. Tissue sections were also evaluated histopathologically. The results demonstrated that Cd exposure could induce release of reactive oxygen species generation, increase levels of TOS and decrease levels of TAS, upregulate mRNA levels of Caspase-3, and induce cell apoptosis. The extracts also prevented the Cd induced increase in AST and GGT levels. Furthermore, the changes of these indicators in OLE treated group was remarkable. The results indicated that exposure to Cd could induce oxidative stress and apoptosis.

Keywords

Cadmium, Olea europaea leaf extract, Caspase

Introduction

Heavy metals occur naturally in the environment but can be elevated through anthropogenic activities such as waste disposal, mining and sludge application (Lucia et al., 2010). Cadmium (Cd) is a pollutant for most human foods because it has a high soil-plant transmission rate, making diet a main source of contact (Satarug et al., 2005). Cadmium can have adverse effects on biota even at environmentally low concentrations (Sinkakarimi et al., 2015). Cd can markedly cause liver injury along its hepatoxic effects (Deniz and Geyikoglu, 2019).

Recently, natural foods and food-derived antioxidants, such as phenolic phytochemicals and vitamins have received considerable attention because they are known to function as chemopreventive agents against oxidative damages (Pérez-Bonilla et al., 2006).

Previous studies have shown that natural antioxidants such as curcumin (Yang et al., 2019), Punica granatum flowers extract (Deniz and Geyikoglu, 2019), grape seed extract (Long et al., 2016), and Pyracantha fortuneana extracts (Ke et al., 2019) can reduce the toxicity of Cd and tissue damage. Like several natural herbs, olive leaves have always drawn much attention, especially for folk medicine. It has a variety of pharmacological properties, including vascular protective effect (Vezza et al., 2019), antioxidant effects (Ayoub et al., 2019) and anti-atherosclerotic effects (Lockyer et al., 2017). Apoptosis represents the physiologic route to eliminate damaged or infected cells in order to maintain tissue homeostasis while avoiding inflammation and damage to the surrounding cells (McIlwain et al., 2013). Death receptor induced apoptosis is mediated by increased both mitochondrial lysosomal permeability and generation of ROS, thus promotes hepatocytes apoptosis in response to toxic materials. Nevertheless, hepatocyte apoptosis, necrosis, formation of ROS and mitochondrial dysfunction occur also in liver toxicity (Guicciardi et al., 2013).

This study aims to investigate the potential protective effects of olive leaf extract (Olea europaea L.) (OLE) administration on acute hepatotoxicity in a rat model of cadmium exposure.

Material and methods

Chemicals

CdCl2 and other chemicals for histological, biochemical analysis were obtained from Sigma (St Louis, MO, USA).

Extraction of O. europaea leaves

Fresh leaves of olive (O. europaea) were directly collected from the olive tree plantation farms from Aljouf region of Saudi Arabia. The leaves were scientifically defined by the herbarium of Biological Sciences Department, Faculty of Sciences,

King Abdulaziz University, Jeddah, Saudi Arabia. The leaves were thoroughly washed and dried at room temperature. The fine quality of dried leaves was kept in dry plastic container until use for extract processes. The method of Al-Attar and Abu Zeid (2013) was used to prepare the extract with some modifications. The aqueous extract of leaves was prepared every two weeks. The dried olive leaves (200 g) were powdered and added to 7 L of hot water. After 3 h, the mixture was slowly boiled for 30 min. After boiling period, the mixture was cooled at room temperature and it was gently subjected to an electric mixer for 20 min. Thereafter the solutions of olive leaves were filtered. Finally, the filtrates were evaporated in an oven at 40 °C to produce dried residues (active principles). With references to the powdered samples, the yield means of leaves extract were 20.3%. Additionally, the extract was stored in a refrigerator for subsequent experiments (Al-Attar et al., 2013).

Experimental animals

Water soluble Cadmium chloride (CdCl2) was used as a source of Cadmium (Cd). In this study, 28 female Wistar Albino rats weighing 280–300 g were used. The rats were obtained from Ataturk University Experimental Research and Application Center. Animals were housed in standard cages under well-regulated conditions (relative humidity range: 45 ± 5%, temperature: 24 ± 1°C and a 12-h light/12-h dark cycle). During the experiment, rats were fed with standard rat diet and water ad libitum. Standard Rat pellets were purchased from Bayramoglu Yem (Erzurum, Turkey, 3.5 % fat, 7.5 % carbohydrates, 23 % protein, 1–2% vitamins and minerals; 3% trace elements, iron, selenium, manganese, zinc, cobalt, iodide, 270 kcal 100 g−1). The experiments were designed and conducted according to ethical norms approved by the Local Animal Care Committee of Ataturk University, Erzurum, Turkey (12.05.2014/54826478-217).

28 rats were randomly divided into 4 groups;

Group 1 (Control): Rats were injected with saline for 5 days

Group 2 (OLE): Rats were injected with 200 mg / kg OLE for 5 days (Atef et al., 2019).

Group 3 (Cd): Rats were injected with 6.5 mg/kg cd for 5 days

Group 4 (Cd + OLE): Rats were injected with 6.5 mg/kg cd + 200 mg / kg OLE for 5 days (Ke et al., 2019).

Blood Sampling

At the end of the experiment, rats were anesthetized, then blood samples were directly collected by cardiac puncture and serum was separated and stored frozen until the biochemical assays. Serum aminotransferases ALT, AST and GGT

activities were determined according to the method of Erel, 2005.

Preparation of tissue homogenates

The tissue samples from each rat were first perfused with phosphate-buffered saline (PBS)/heparin and frozen tissues were homogenized in a TissueLyser II grinding Jar Set (Qiagen, Hilden, Germany). Approximately 100 mg of ground tissue was homogenized in 1 ml PBS homogenate buffer in an eppendorf tube with TissueLyser II, and the samples were then centrifuged.

Measurement of tissue total antioxidant status and total oxidant status levels

TOS and TAS from each sample supernatant was measured via colorimetric methods by using commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey). The results of the TAS (Erel 2004) and TOS (Erel 2005) in the tissues were expressed as mmol/mg protein, μmol/mg protein, respectively

Histological Analysis

Liver tissues were fixed in 10% neutral formalin and routinely processed in paraffin. Liver tissues were also trimmed into cassettes, dehydrated in graded ethanol solutions, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm for hematoxylin and eosin (H&E) staining were prepared prior to microscopic analysis.

Statistical Analysis

The differences in variance were analyzed statistically using a one-way analysis of variance (ANOVA) test by Graphpad prism 5.0 statistics software (GraphPad, La Jolla, CA, USA). Tukey's test was used as a post hoc.

Results

Differences of liver parameters

The mean values of ALT and AST were significantly higher in Cd treated rats than the control group ($P < 0.001$) as shown in Figure 1 and 2. The GGT level of the control group and Cd treated rat was not associated significantly (Data not shown).

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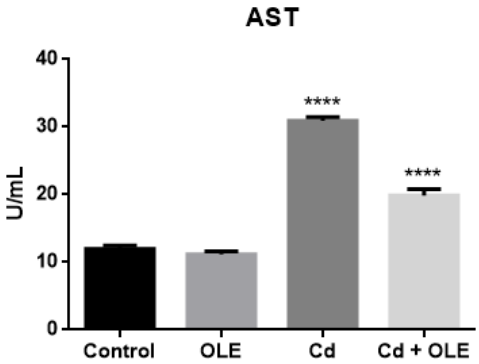

Figure 1. Modulatory effect of OLE on serum AST levels in rats exposed to cadmium chloride. Data are presented as mean ± Standard Error of Mean (SEM) (n = 7). * denotes significant differences between other studied groups and control (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****:

$p < 0.0001$),) by Tukey's multiple range tests.

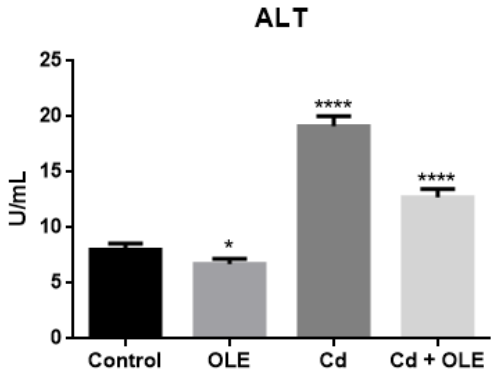

Figure 2. Modulatory effect of OLE on serum ALT levels in rats exposed to cadmium chloride. Data are presented as mean ± Standard Error of Mean (SEM) (n = 7). * denotes significant differences between other studied groups and control (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$),) by Tukey's multiple range tests.

In Figure 3 and Figure 4, the levels of TAS and TOS are shown as quantified in serum. The level of TAS diminished significantly due to the administration of Cd (6.5 mg/kg) compared with the control and OLE group (200 mg/kg) ($p < 0.0001$) (Figure 3). Liver TOS levels were significantly raised in Cd treated group compared to control and OLE groups ($p < 0.0001$) (Figure 4).

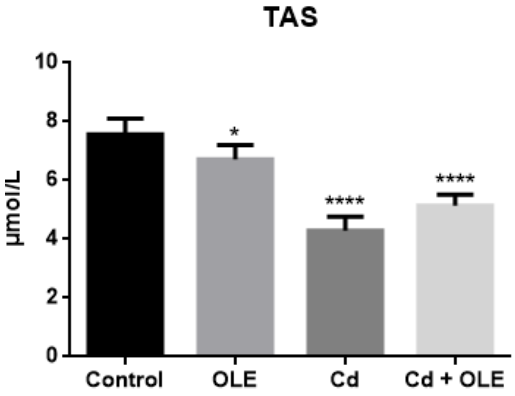

Figure 3. Modulatory effect of OLE on liver TAS levels in rats exposed to cadmium chloride. Data are presented as mean ± Standard Error of Mean (SEM) (n = 7). * denotes significant differences between other studied groups and control (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$),) by Tukey's multiple range tests.

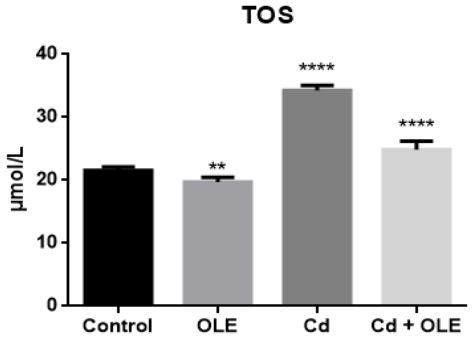

Figure 4. Modulatory effect of OLE on liver TOS levels in rats exposed to cadmium chloride. Data are presented as mean ± Standard Error of Mean (SEM) (n = 7). * denotes significant differences between other studied groups and control (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$),) by Tukey's multiple range tests.

Hepatic injury induced by Cd

As shown in Figure 5C, the typical pathological changes were observed in the liver of the rat treated with Cd. The hepatic parenchyma close to the lesions was progressively invaded by fibrous connective tissue septa, and solitary islands of hepatic tissue were observed. At the same time points, no hepatic injury was observed in the rats of the control group and OLE group (Figure 5A and 5B). OLE partially cured liver damage caused by Cd (Figure 5D).

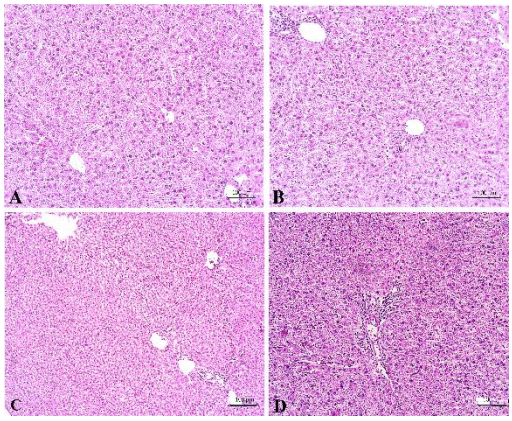

Figure 5. Illustrative photographs from the liver showing the protective effect of OLE on cadmium-induced hepatotoxicity in rats. (A) Control rat liver and (B) OLE treatment rat liver (H&E × 10 eyepiece magnification,) sections displaying normal hepatic parenchyma. (C) Cadmium (6.5 mg/kg) treated rat liver (H&E × 10 aeyepiece magnification) section showing extensive degeneration of hepatocytes with focal necrosis, vacuolated cytoplasm, inflammatory cell infiltration, and damaged central vein. (D) Cadmium (6.5 mg/kg) + OLE (200 mg/kg)-treated rat liver (H&E × 10 eyepiece magnification, respectively) section exhibiting normal appearance of hepatocytes with mild sinusoidal dilation. H&E hematoxylin and eosin staining, scale bar = 100 μm

Caspase 3 activation, a marker of the apoptotic protease cascade, was measured by immunohistochemistry. There was weakly caspase 3 expression in the control and OLE group rat livers (Figure 6A and 6B), whereas Cd treated group showed increased caspase 3 expression (Figure 6C). OLE decreased caspase 3 expression compared to Cd group (Figure 6D). There was a significant difference between only Cd treated and Cd + OLE treated rats.

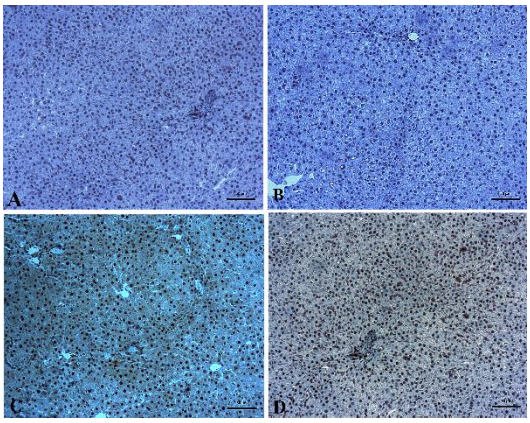

Figure 6. Caspase 3 immunohistochemistry expression among the examined groups. (A) Control rat liver and (B) OLE treated rat liver showing very occasional weak nuclear and cytoplasmic foci expressing. (C) Cadmium (6.5 mg/kg) treated rat section showing apoptotic hepatocytes condensation with plenty of nuclear and patchy cytoplasmic. (D) Cadmium (6.5 mg/kg) + OLE (200 mg/kg)-treated rat liver section exhibiting caspase 3 expression in infiltrating lymphocytes and in hepatic cells.

Discussion

Environmental contamination by Cd is a worldwide problem. Cd is a highly toxic heavy metal and its toxicity occurred by

ingestion and inhalation (Satarug et al., 2010). In this study, OLE experimented for overcoming the Cd toxicity in liver of rats. Serum ALT levels were elevated significantly in Cd group. Similarly, our results come in the same line with in a study conducted on Cd toxicity in rats (Toppo et al., 2015). The data of the present study pointed out that; there was marked an increase in AST and ALT, this might be due to Cd-induced oxidative stress leading to high level of H_2O_2 which in turn causing impairment in lipid metabolism and lipid peroxidation which is correlated with Cd toxicity. The rats treated with OLE alone showed no significant effect in liver functions. Significant restoration of hepatic enzymes was observed, so OLE contributing hepato-protection against Cd toxicity. However, the obtained results confirmed that the OLE was very active against the ROS. The result of current study showed that the elevation of the liver enzyme test (ALT and AST) was significantly associated with Cd compared with the control group. Therefore, liver enzyme tests have a positive role in the management of Cd toxicity.

In addition, to understand the effect of Cd on liver oxidative stress in rat and the antioxidant effect of OLE on Cd-induced oxidative stress, total antioxidant (TAS) and total oxidative stress (TOS) statuses were established in the liver. In our study, Cd on the hepatic tissue of normal rats showed a significant increase ($p < 0.001$) in the level of TOS associated with a noticeable decrease ($p < 0.001$) of TAS level, compared with their respective values in the liver of control rats (Figure 4 and 5). Likewise, a recent study demonstrated that TAS and TOS levels were significantly altered by Cd administration in rats (Bahri et al., 2019).

In this paper, this study was conducted to observe the composition changes of liver tissues after rat were injected with cadmium. No cases of death or abnormal pathological signs were observed in either Cd or OLE groups during the experiments. Histopathologic findings of OLE administration did not show obvious differences between the control group (Figure 5B). However, significant changes were observed in Cd group (Figure 5C). Treatment of rats with 6.5 mg/kg of Cd caused changes in the liver including extensive degeneration of hepatocytes with focal necrosis, vacuolated cytoplasm, inflammatory cell infiltration, and damaged central vein (Figure 5C). According to this study, Cd

+ OLE administration significantly alleviated tissue damage in comparison with the Cd group (Figure 5D) and this is similar to that in Ağır and Eraslan (Ağır et al., 2019).

Cd increased the mRNA expression of apoptosis executioner caspase 3 and initiated the apoptosis in rats. Compared with the control group, the caspase 3 expresion were significantly increased in Cd group (Figure 6C). Compared with the Cd group, the expresion of caspase 3 was decreased in the Cd + OLE group (Figure 6D). This result was consistent with our previous study (Deniz and Geyikoglu, 2019).

It is concluded that OLE therapy had beneficial effect on the normal hepatic tissues. In turn, Cd-treated liver showed a negative modulation in the metals and redox state. In addition, OLE exhibited a pronounced therapeutic effect in mitigating the histological changes in the studied tissues. Thereby, OLE could be used as an adjuvant treatment post-exposure to Cd.

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