Olive leaves’ extract may attenuate cadmium-induced liver damage in Wistar rat

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This study investigates the possible attenuation of cadmium toxicity by using fresh aqueous olive leaves extract (OLE) of *Olea europea*. Wistar rats were divided into a control group received a standard diet, two positive controls received 0.25 g/kg BW (OL1) and 0.5 g/kg BW (OL2), one group treated with CdCl₂ (40 mg/kg BW), and finally, two other groups supplemented with the combination of Cd and OL (Cd+OL1, Cd+OL2). Cadmium and OL were administrated daily by gavage for one month. Hepatic histology, malondialdehyde (MDA), reduced glutathione (GSH), and serum biomarkers were evaluated. Results indicate a significant increase in the MDA level of the Cd group compared with the three control groups, however, a significant decrease was noted in the groups of Cd+OL1 and Cd+OL2 compared to the Cd group. For the GSH, the Cd group showed a significant decrease compared to all control groups. A significant rise in the concentration of total bilirubin, total cholesterol, and triglycerides and in the activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase was observed in the Cd-exposed rats compared to all controls, but the level of albumin and total proteins manifested significant decrease. However, the combined treatments have attenuated the toxicity of Cd through the recorded significant changes of most studied biochemical markers. The above results were confirmed by the histological study which revealed certain liver alterations in the Cd-exposed rats, while the co-administration of OL has reduced the hepatic tissue disorganization. In conclusion, OL seems effective to diminish the prooxidative effect of cadmium.

Key words: Attenuation, Cadmium, olive leaves extract, biochemical markers, rat
Environmental contaminants, including heavy metals, are responsible for the generation of oxidative stress. Cadmium is a pollutant widely originated from human activities as battery industries, pigments, plastics and cigarette smoke. It is an element easily distributed in all environmental compartments; water, air and soil (Lauwerys, 1999), particularly after prolonged exposure (Julin et al., 2012), threatening the health of biota. It causes pathophysiological disorders in vital organs as liver, kidneys, brain, testes and bone, as well as in the biochemical and the hematological markers (Grosicki and Kowalski, 2002).

Cadmium is transported via the blood, which could accumulate in erythrocytes bound to hemoglobin (Nordberg et al., 2007) and to high molecular weight plasma proteins as albumin. It is rapidly distributed mainly to the liver and kidneys where it induces the synthesis of metallothioneins, which have a high affinity for trace metals, due to its richness in sulfhydryl groups (Masson, 1999). The existence of Cd in organisms can lead to an increase in reactive oxygen species (Oh et al., 2006), that are likely to cause oxidative damage to biological macromolecules. In addition to oxidative stress, apoptosis, inflammation and competitive suppression of zinc may be implicated in these mechanisms (Adedara and Farombi 2010; Seok et al., 2006; Amara et al., 2008; Dorostghoal et al., 2017). Cadmium can affect energy processes, membrane transport, protein synthesis, and genetic structure (Hartwig and Schwerdtle, 2002).

The use of natural products for pollutants’ toxicity mitigation is a growing field of research, because these products are generally available locally, easy to use, cheap and usually without adverse effects. Basing on this, cadmium toxicity can be neutralized by natural components that often found in the plant kingdom (Bhattacharya, 2018). Recently, they have been recognized as a valuable source of antioxidant compounds because of their free radical scavenging ability and to their benefits to human health (Cazzola and Cestaro, 2014; Manach et al., 2018). The Mediterranean diet rich in olive products has always been associated with human health since ancient times. Olive leaves are widely used in traditional remedies as antioxidant (Sedef and Karakaya, 2009), anti-inflammatory (Miles et al., 2005), anti-hyperlipidemia (Andreadou et al., 2006), anti-diabetic (Hedya et al., 2009), and in the treatment of many diseases (Szychlinska et al., 2019), for the reason that it contains several potentially bioactive elements rich in phenolic compounds including flavones, flavonols and catechins (Özcan and Matthaüs, 2017).

Accordingly, this work is based on studying the possible detoxification capacity of fresh aqueous olive leaves extract (Olea europea) against the cadmium induced toxicity on male Wistar rat by measuring certain serum biochemical markers and the hepatic histological profile after one-month experimental trial. Thus, OLE and Cd were administered together by gavage not only to attenuate Cd at the cellular level but also to attain a possible interference with Cd uptake in the digestive tract to reduce its bioavailability since certain food is contaminated with this toxic metal.

MATERIALS AND METHODS

Animals’ rearing

Male Wistar rats were obtained from Pasteur Institute (Algiers), weighing 241±7 grams. Animals were maintained in the Animal House of the Biology Department under controlled conditions, in which they subjected to standard experimental conditions of light, humidity and temperature. Standard diet was supplied by the “ONAB, rodent feed” from Bejaia, Algeria and water were available ad libitum.

Preparation of the olive leaves’ extracts

Medium size fresh olive leaves were harvested during October from the region of Annaba, and then washed with distilled water to remove dust. A known quantity (0.25g and 0.5g) of leaves was homogenized daily by an electric mixer with the addition of a sufficient volume of distilled water and then filtered to attain an aqueous olive leaves extract (OL), sufficient to offer 1 ml/rat.

Animals’ treatment

Sixty male rats were equally divided into eight groups. The control received standard diet, the two positive groups represented by the OL1 (0.25g/kg bw) and the OL2 (0.5g/kg bw), in addition to the cadmium
group (40 mg CdCl$_2$/kg bw). The other three groups were treated with a combination of cadmium and olive leaf extracts (Cd+OL1) and (Cd+OL2). CdCl$_2$ of the Cd group was dissolved in distilled water, while CdCl$_2$ of the combination groups was dissolved in the filtered OL. All administrations were given daily in the morning by gavage for a period of one month. Animals’ treatments were authorized by the Ethical Committee of Animal Sciences at the University of Badji Mokhtar-Annaba, before starting the experimental work.

Estimation of Malondialdehyde

Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) to produce a red colored complex that has a peak absorbance at 532 nm. Therefore, 125 μL of supernatant were homogenized by sonication with 50 μL of PBS and 125 μL of trichloroacetic acid-butylhydroxytoluene (TCABHT) to precipitate proteins, which was then centrifuged at 1,000 rpm for 10 min, at 4°C. After that, 200 μL of supernatant was mixed with 40 μL of HCl (0.6 M) and 160 μL of TBA dissolved in Tris, and then the mixture was heated at 80 °C during 10 minutes, in which the supernatant obtained was read at 530 nm. The amount of TBARS was calculated (nmol GSH/mg proteins) using a molar extinction coefficient of $1.56 \times 10^5$ M/cm (Buege and Aust, 1984).

Estimation of reduced glutathione

The measurement of GSH concentration was based on the development of a yellow colour after adding DTNB to compounds having sulfhydryl groups (Ellman, 1959). Thus, 200 μL of 0.25% sulphasalicylic acid was added to 800 μL of organ supernatant, and then the mixture was centrifuged at 1,000 rpm for 15 minutes. After that, 500 μL of the obtained supernatant was mixed with 1,000 μL phosphate buffer (0.1 M, pH 7.4) and 25 μL of DTNB (10 mM). Total GSH content (nmol GSH/mg proteins) was calculated at 412 nm.

Protein assay

The protein content of supernatant was measured according to the Bradford method by using bovine serum albumin as standard (Bradford, 1959).

Biochemical assays

At the end of the experimental period, all animals were sacrificed by cervical decapitation and blood samples were collected in dry tubes, centrifuged at 3000 rpm for 10 minutes to obtain serum. Afterward, alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP), albumin, total proteins, total bilirubin, total cholesterol and triglycerides were measured by using an automatic biochemistry analyzer (ARCHITECT ci4100) supplied with commercial kits (Spinreact, Spain).

Histological examination

A fraction of the liver tissue was fixed in 10% formal and proceeds to routine histopathological procedure. Then, water was removed by using ethanol (70~100%), and then it was embedded in a paraffin baths at 58°C. Sections of 4~6 μM were prepared using a paraffin blocks using a Leica Rotary Microtomy (Wetzlar, Germany). The sections were then stained with Hematoxylin-Eosin (H-E). Slides were studied and photographed under alight microscope (Leica DM 500, Leica light microscope, Wetzlar, Germany).

Statistical analysis

Results were expressed as Mean ± SD. Data were analysed by one-way analysis of variance (ANOVA). The Graphs were drawn by Pad Prism 5.0 software (Graph Pad software, Inc., San Diego, USA). Results were considered as statistically significant at $P \leq 0.05$.

RESULTS

Serum biochemical markers

Serum biochemical markers are seen in table 1. Significant increase of ALT, AST and ALP activity in the Cd group compared to the control and to the two positive controls, with a significant decrease of the groups received the co-administration of olive leaves compared to the Cd group were observed, with the exception of ALT of Cd+OL1.

Hepatic GSH and MDA

The level of reduced GSH has decreased significantly in animals of the Cd group compared to the control, OL1 and OL2 (Fig. 1). The Cd+OL2 group was significantly different than that of the Cd group. However, there was a significant increase in MDA level of the Cd group compared to the three controls; nevertheless the two combined treatments (Cd+OL1 and Cd+OL2) were significantly decreased compared to the Cd group (Fig. 2).
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Figure 3: The histological profiles of rat hepatic tissues of the control (x400s), the positive controls OLE1, OLE2 (x400), the Cd group (x100 and x400) and the combined groups of Cd+OLE1 and Cd+OLE2 (x400) after 30 days of experimental trial (stained with H&E). NH: Normal hepatocytes; CV: Central vein; PV: Portal vein; Cong: Congestion; Dgt: Degeneration; Inf: Inflammation by leucocytes; BD: Bile duct; Nc: necrosis.
Total bilirubin concentration has been raised in the Cd treated group, and even in the combined groups. Albumin level was significantly higher in the Cd group compared to the control and to the positive controls. The co-administered groups were not statically different than that of the Cd group.

A significant decrease in total protein concentration was observed in animals of the Cd group compared to all controls, with a significant augmentation in the groups treated with Cd+OL2 compared to the Cd group.

The concentration of total cholesterol and triglycerides of Cd group have increased significantly than those of the three controls. Though, the co-administration of olive leaves extracts caused significant decrease only in the levels of total cholesterol when compared to the Cd group.

**Hepatic histology**

The hepatic histology of the different groups is shown in fig 12. The positive controls of olive leaves extract are similar to the control group. However, the group treated with CdCl₂ is characterized by the appearance of certain disorganization, vein congestions, leucocytic cells infiltration, tissue degeneration, and increased widening of sinusoids. The combination of Cd+OL has led to a reduced degeneration and inflammation of the parenchyma with moderate disorganization of liver tissue.

**DISCUSSION**

Given the recorded results, it was noted that the two doses of positive controls of fresh olive leaf extracts did not affect the level of all the biological markers during the four-week experimental work. The OL and Cd were administered together by gavage owing to interfere with Cd uptake in the digestive tract.

The mentioned decline in hepatic GSH level of male rats when exposed to cadmium for one month is in line with other previously published works (Zaidi et al., 2004; Fotakis and Timbrell, 2006; Nampoothiri and Gupta, 2008; Renugadevi and Prabu, 2010; Mohamed, 2019). Thus, rats subcutaneously injected with cadmium for one month had caused a depletion of total thiols and total antioxidant levels, in addition to the enzymatic and non-enzymatic antioxidants (Sanjeev et al., 2019). The anti-cadmium cellular defense is based mainly on the metal-sensitive transcription factor (MTF1), especially metallothioneins, and on the level of glutathione (Wimmer et al., 2005). In addition, the deficiency of the antioxidant defense system is considered a critical event in the induction of hepatotoxicity by cadmium. Glutathione plays its antioxidant role also in synergy with antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase (Morin et al., 2004). This metal can also bind with the GSH thiol group and the metallothionein that play a major function in the intracellular detoxification of trace metals (Stohs et al., 2000). The administration of olive leaf extract to Cd-exposed rats in this study resulted in a significant elevation of hepatic GSH levels, just like the results published elsewhere (Alirezai et al., 2012). The protective action of olive leaves may occur because of the potent activity of scavenging free radicals; this antioxidant property is due to the presence of polyphenols as hydroxytyrosol and oleuropein and tocopherol (Savournin et al., 2001). It is noted that flavonoids and phenolic compounds can chelate metals and potentiate body cadmium clearance (Heim et al., 2002). All enzymes involved in the biotransformation of xenobiotics have the potential to generate reactive intermediates, most of which are detoxified by glutathione. The latter is also a co-factor of glutathione peroxidase (GPX), which plays an important role in protecting cells against lipid peroxidation. It has been reported that olive leaves contain many potentially bioactive compounds that can have antioxidant effects (El and Karakaya, 2009).

In the current study, a significant increase in hepatic MDA content was observed in 30-day Cadmium-exposed rats, leading to worsening of lipid peroxidation. Accordingly, this result is consistent with several previous works cited (Nampoothiri and Gupta, 2008; Renugadevi and Prabu, 2010; Mohamed, 2019; Manna et al., 2008; Ognjanovic et al., 2008; Ghorbel et al., 2017). Cadmium may produce oxidative damage in the liver by increasing membrane lipid peroxidation (Athmouni et al., 2018). It indirectly generates various radicals such as superoxide, hydroxyl and nitric oxide, thus causing damage compatible with oxidative stress.
Thus, free radicals attack cells, leading to destabilization and disruption of the cell membrane, resulting in tissues’ MDA accumulation. In the combined groups, the addition of olive leaves’ extract have significantly reduced MDA concentrations in liver tissues. Similarly, (Botsoglou et al., 2013) postulated that the administration of α-tocopherol, one of the olive leaf compounds, improved the antioxidant status of eggs enriched in fatty acids because the laying hens received a diet supplemented with 200 mg/kg of α-tocopherol, 5g or 10g olives/kg food. Polyphenols, the main compounds of olive leaves, also was able to minimize the harmful effects of lipid peroxidation by lowering the MDA concentration (Kamboh and Zhu, 2013) as that of oleuropein, which prevented lipid cell membranes from oxidation (Ferreira et al., 2007).

Current results show an increase in the enzymatic activity of ALT, AST and ALP, as well as the total serum bilirubin of rats exposed to cadmium. Such results are in line with other published works concerning cadmium toxicity in rats (Mohamed, 2019; Albasha and Azab, 2014; Adefegha et al., 2015; Dardouri et al., 2016). The increase in these biomarkers indicates hepatic injury that is explained by the leakage of enzymes from the tissue to the blood due to the alteration of membrane permeability (Navarom et al., 1993). Thus, liver homogenate was found to contain low activity of ALT, AST and ALP in rats exposed orally to cadmium (Adefegha et al., 2015). In many organs, cell damage is determined by the presence of certain cytoplasmic enzymes in the blood as the case after cadmium accumulation in the hepatocytes. It has been suggested that bile impairment may cause elevation of serum alkaline phosphatase (Moss and Butterworth, 1974) and serum total bilirubin (Liss et al., 1985; Ajlore et al., 2012). The combination of olive leaf extracts with cadmium has significantly improved the enzymatic activities of ALT, AST and ALP, but not the level of bilirubin, where the antioxidants present in this plant can stabilize hepatocellular membranes and protect the molecules against the toxic effects of cadmium. In addition, consumption of olive leaf extract at 3% and 6% to diabetic rats resulted in a reduction in the activity of liver ALT and AST (Mousa et al., 2014). Similar results were reported, after oral administration of the olive leaf extracts (0.1, 0.25 and 0.5 g/kg body weight) for 14 days, which significantly decreased the activity of AST and ALT (Eidi et al., 2009).

The significant decrease of serum total proteins and albumin in rats was due to cadmium, which may be explained by liver and/or renal damage, via reducing the synthesis in the first (Chawla, 2003) and the defect of filtration in the second (Adefegha et al., 2015). Therefore, the exposure of rats to cadmium has decreased serum total proteins (Adefegha et al., 2015; Sanjeev et al., 2019), altered the plasma protein profile (Adefegha et al., 2015), and reduced plasma albumin level (El-Demerdash, 2004). In these circumstances, only the higher dose of olive leaves’ extract coadministration has helped to stabilize the total protein levels to a normal level in rats after one-month cadmium exposure, while that of albumin was still remarkably lower even in both co-administered groups. Renal glomerular dysfunctions were occurred in workers exposed to cadmium for a long period reflected by an increase in the excretion of certain proteins including albumin (Buchet et al., 1979).

According to the results obtained, an increase in lipid markers was recorded for the cadmium group, which is probably due to the degradation of adipose tissue by altering energy and lipid metabolism (Chen et al., 2018). However, the combination of olive leaf extract with cadmium produced normal levels in these parameters. There is, therefore, protection against cadmium intoxication obtained by the antioxidant supplementation of the extract. Accordingly, the olive leaf extract was confirmed to have antioxidant properties (Cheurfa et al., 2018), and lower human plasma total cholesterol, LDL-cholesterol and triglyceride levels, as cardiovascular risk factor (Lockyer et al., 2017). The polyphenol hydroxytyrosol, is an exceptional free radical scavenger (Fernandez-Bolanos et al., 2008), that is found in olive leaves, which inhibits the oxidation of human LDL easily absorbed by the gastrointestinal tract. In addition, oleuropein, the main polyphenolic compound of olive leaf was reported to exert a strong antioxidant activity (BenSalah, 2012). Further, the oleuropein was confirmed to chelate metal ions, responsible for free radical generation (Andrikopoulo et al., 2002). Furthermore, olive leaf
extracts at 0.1, 0.25 and 0.5 g/kg body weight were able to reduce the level of blood total cholesterol and triglycerides after 14 days in normal and diabetic wistar rat (Eidi et al., 2009).

The histological profiles of the hepatic parenchyma of the control group and the positives controls are well organized, indicating a normal healthy tissue. The administration of Cd resulted in certain hepatocyte disorganization, inflammatory cells infiltration with vascular lesions leading to the formation of many degenerative structures; such effect is in line with that carried out on mice (Ersan et al., 2008; Gong et al., 2008). The variations observed in the liver architecture in the cadmium treated rats is likely induced by the formation of ROS by cadmium ions, the later are responsible on the cell membrane damage and consequently on the degeneration of hepatic parenchyma (Ognjanovic et al., 2008). Moreover, it was confirmed that chronic cadmium administration to small rodents including rat is mainly accumulated in the liver and the kidney, and provokes certain biochemical and functional changes to target organs (Swiergoszkowalewska, 2001). For the groups received the combination of cadmium and olive leaves aquatic extract, the histological sections showed a considerable improvement with slight degenerations of the hepatocytes and decreased widening of blood sinusoids. Such results are comparable to those reported earlier in which cadmium intoxicated rats were treated with naringenin (Ognjanovic et al., 2008); a phenolic component of olive leaves (Olmo-García et al., 2018). 

**CONCLUSION**

The observed disturbance of the major biochemical markers is a strong indication of cadmium accumulation in the target organ since it is the main site of xenobiotics’ detoxification. However, cadmium toxicity was weakened by the co-administration of olive leaves’ extract, through the normalization of most markers, and the protection of hepatic histological integrity to some extent. Olive leaves aqueous extract appears as a promising source of bioactive compounds.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interests.

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