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ORIGINAL ARTICLE

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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 $CdCl_2$ (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 mechanismsn (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, flavonolsand catechins (Özcan *and* Matthäus, 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 × 105 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% sulphosalicylic 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% formol 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 from 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 andCd+OL2) were significantly decreased compared to the Cd group (Fig. 2).

Table 1: The levels (mean ±SD) of ALT (U/L), AST (U/L), ALP (U/L), Total bilirubin (mg/dl), Albumin (g/L), total proteins (g/L), total cholesterol (g/L) and triglycerides (g/L)in rats exposed to cadmium and co-administered with olive leaves' extracts after 30 days of experimental trial.

Markers	Control	OL1	OL2	Cd	Cd+OL1	Cd+OL2
ALT	59±6.10^{a}	56.74±8.69^{a}	57.56±7.90^{a}	105.13±17.2^{b}	84.9±13.9^{b}	69.17±12.3^{a}
AST	204±13.82^{a}	196±10.14^{a}	186±11.25^{a}	259±15.40^{b}	202±12.30^{a}	193±13.37^{a}
ALP	160±9.96^{a}	157±11.20^{a}	155±14.43^{a}	428±29.68^{b}	277±12.96^{c}	253±16.76^{c}
T. bilirubin	0.64±0.072^{a}	0.671±0.068^{a}	0.625±0.063^{a}	0.77±0.088^{b}	0.71±0.07^{b}	0.73±0.08^{b}
Albumin	37±2.51^{a}	35±2.912^{a}	35.90±2.23^{a}	27.15±3.87^{b}	29.46±2.73^{b}	30.52±3.62^{b}
T. proteins	76±5.80^{a}	71.72±4.872^{a}	73.47±5.151^{a}	52.98±6.90^{b}	63.89±6.98^{b}	71±4.546^{a}
T. choleterol	1.045±0.037^{a}	1.00±0.067^{a}	0.99±0.06^{a}	1.69±0.081^{b}	1.34±0.051^{c}	1.13±0.022^{d}
Triglycerides	0.437±0.029^{a}	0.42±0.034^{a}	0.405±0.049^{a}	0.566±0.06^{b}	0.46±0.06^{a,b}	0.47±0.07^{a,b}

OL1: Olive leaves extract 0.25g/Kg bw; OL2: Olive leaves extract 0.5g/Kg bw; Cd: $CdCl_2$ (40mg/Kg bw). Groups having different superscript letters are significantly different.

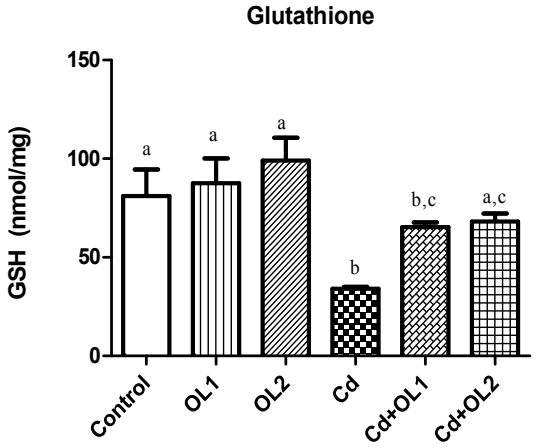

Figure 1: Hepatic glutathione level (Mean ± SD) in rats exposed to cadmium and co-administered with olive leaves' extract after 30 days of experimental trial. OL1: Olive leaves extract 0.25g/Kg bw; OL2: Olive leaves extract 0.5g/Kg bw; Cd: $CdCl_2$ (40mg/Kg bw).
Groups having different superscript letters are significantly different.

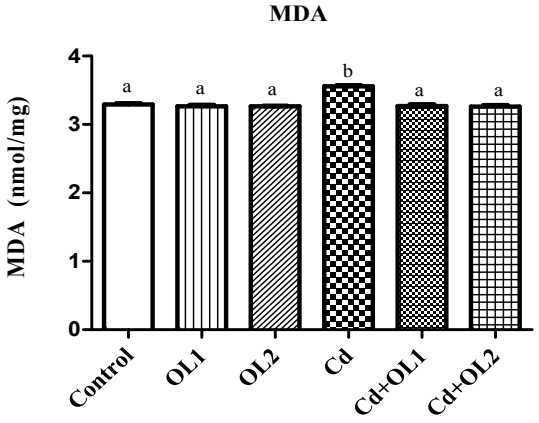

Figure 2: Hepatic MDA level (Mean ± SD) in rats exposed to cadmium and co-administered with olive leaves' extracts after 30 days of experimental trial. Doses and statistics are the same as in Fig 1.

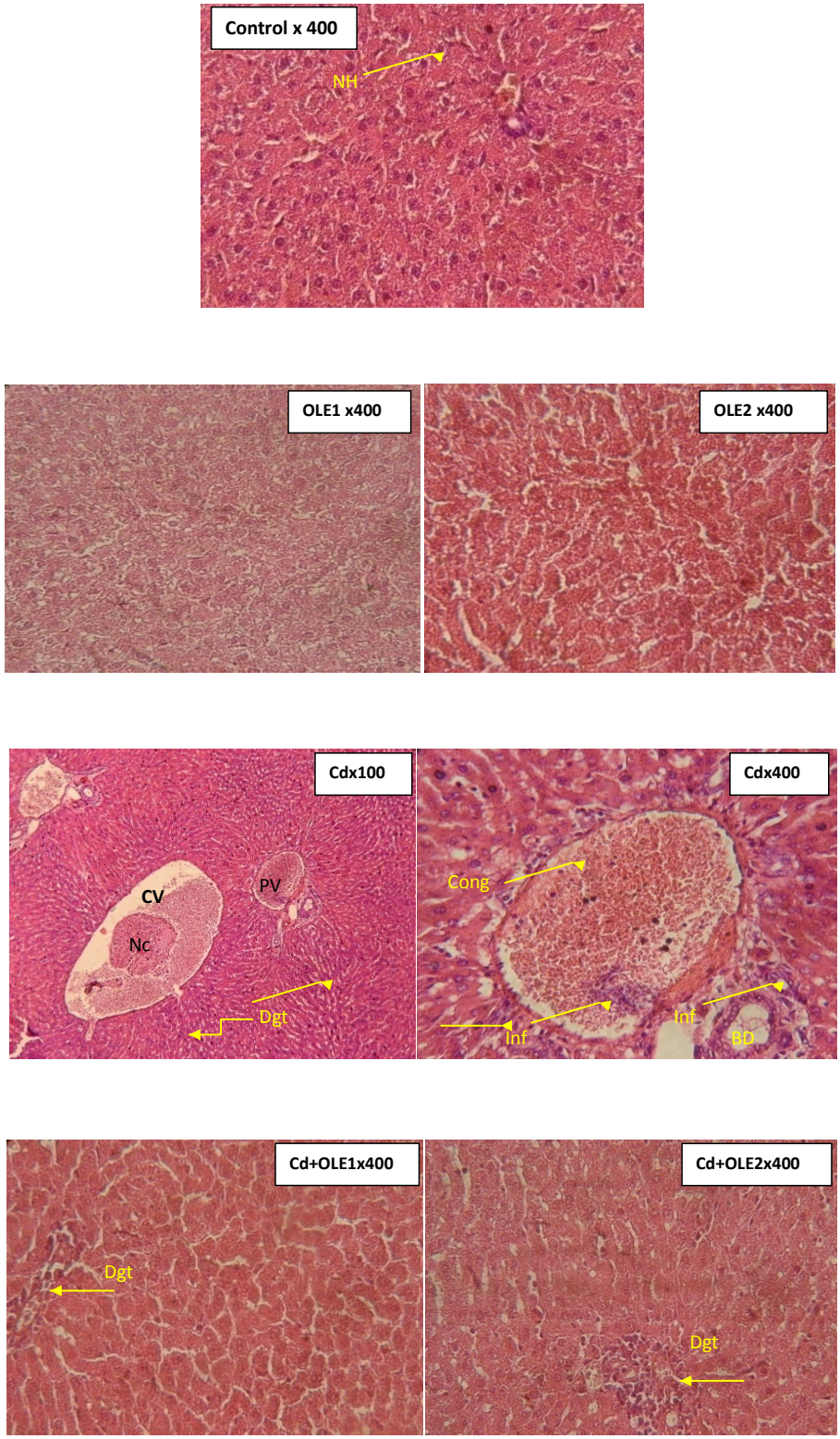

Figure 3: The histological profiles of rat hepatic tissues of the control (x400≤), the positive controls OLE1, OLE2 (x400), the Cd group (x100 and x400) and the combined groups of Cd+COLE1 and Cd+OLE2 (x400) after 30 days of experimental trial (stained with H&E). NH: Normal hepatocytes; CV: Central vein; 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-administred 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 augmentationin 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 $CdCl_2$ 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 *and* Banu, 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 groupand 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 (Alirezaei *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 (EI *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

(Stohs *et al.,* 2000). 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; Ajilore 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, oleoropein, 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 (Swiergosz-Kowalewska, 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). *Recently, methanolc olive leaf extract was confirmed to be efficient in protecting liver cells from cadmium induced injuries* (Albasher, 2018). Furthermore, kidney tissue damage provoked by cadmium were prevented by the co-adminastration of olive leaves aqueous and ethanolic extracts (Ranieri *et al*., 2019) and also by oleuropein supplementation (Jemai, 2019). *Finally,* olive leaves of *Olea europaea* tree is believed to have a potential beneficial effects on human health (EI and Karakaya, 2009), because of polyphenols which have a strong antioxidative and chelating outcome on cadmium toxicity (Mężyńska *and* Brzóska, 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.

REFERENCES

Adedara I.A., Farombi E.O. (2010). Induction of oxidative damage in the testes and spermatozoa and hematotoxicity in rats exposed to multiple doses of ethylene glycol monoethyl ether. *Hum. Exp. Toxicol.*, **29**,801-812.

Adefegha S.A., Omojokun O.S., and Oboh G. (2015). Modulatory effect of protocatechuic acid on cadmium induced nephrotoxicity and hepatoxicity in rats in vivo. *SpringerPlus*, **4**, 619.

Ajilore B.S., Atere T.G., Oluogun W.A. et al. (2012). Protective effects of *Moringa oleifera* Lam on cadmium induced liver and kidney damage in male Wistar rats. Int. J. Phytother. Res., **2(3)**,42–50.

Albasha M.O. and Azab A.E. (2014). Effect of cadmium on the liver and amelioration by aqueous extract of fenugreek seeds, rosemary and cinnamon in guinea pigs: histological and biochemical study. *Cell Biol.,* **2(2)**, 7–17.

Albasher G. (2018). Anti-fibrogentic and

hepatoprotective potential of methanolic olive extract on cadmium induced toxicity in rats. *Life Sci.*, **15(7)**, 1-11.

Alirezaei M., Dezfoulian O., Kheradmand A., Neamati S., Khonsari A. and Pirzadeh A. (2012). Hepatoprotective effects of purified oleuropein from olive leaf extract against ethanol-induced damages in the rat. *Iran. J. Veter. Res*., **13(3)**, 218-226.

Amara S., Abdelmelek H., Garrel C., Guiraud P., Douk T. and Ravanat J.L. (2008). Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. *J. Reprod. Develop*., **54**, 129-134.

Andreadou I., Iliodromitis E.K., Mikros E., Constantinou M., Agalias A., Magiatis P., et al. (2006). The olive constituent uropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. J. Nutr. **136(8)**, 2213-2222.

Andrikopoulo N.K., Kaliora A.C., Assimopoulou A.N. and Papageorgiou V.P. (2002). Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against in vitro low-density lipoprotein oxidation. J. Med. Food, **5**, 1–7.

Athmouni K., Belhaj D.M., Kadmini H.K., El-Feki A., and Ayadi H. (2018). *Arch. Physiol. Biochem.*, **124(3)**, 261-274.

BenSalah M., Abdelmelek H. and Abderraba M. (2012). Study of phenolic composition and biological activities assessment of olive leaves from different varieties grown in Tunisia. Med. Chem., **2(5)**, 107–111.

Bhattacharya S. (2018). The role of medicinal plants and natural products in melioration of cadmium toxicity. *Orient. Pharm. Exp. Med*. **18(10)**, 177-186.

Botsoglou E., Govaris A., Fletouris D. and Iliadis S. (2013). Olive leaves *(Olea europea L.*) and α-tocopheryl acetate as feed antioxidants for improving the oxidative stability of α-linolenic acid-enriched eggs. *J. Anim. Physiol. Anim. Nutr*., **97(4)**, 740-753.

Bradford M. (1976). A rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., **72**, 248-254.

Buchet J.P., Roels H., Masson P. and Lauwerys R. (1979). Renal excretion of proteins and enzymes in workers exposed to cadmium. *Europ. J. Clin. Invetig.*, **9(1)**, 11-22.

Buege J.A. and Aust S.D. (1984). Microsomal lipid peroxidation. *Methods. Enzymol*., **105**, 302-310.

Cazzola R. and Benvenuto C. (2014). Diabetes: Oxidative Stress and Dietary Antioxidants Chapter 9, Antioxidant Spices and Herbs, Used in Diabetes, Elsevier.

Chawla R. (2003). Practical clinical biochemistry: methods and interpretations. Jaypee Brothers Publishers, New Delhi.

Chen S., Zhang M., Bo L. et al. (2018). Metabolomic analysis of the toxic effect of chronic exposure of cadmium on rat urine. *Environ. Sci. Pollut. Res*., **25(4)**, 3765.

Cheurfa M., Abdallah H.H., Allem R., Noui A., Picot-Allain C.M.N. and Mahomoodally F. (2018). Hypocholesterolaemic and antioxidant properties of *Olea europaea L*. leaves from Chlef province, Algeria using in vitro, in vivo and in silico approaches. *Food Chem. Toxicol*., **123**, 98–105.

CRL E.H.N. (2003). Cadmium Review. Nordic Council of Ministers. Report no 1(4).

Dardouri K., Haouem S., Gharbi I., Sriha B., Haouas Z., El Hani A. and Hammami M. (2016). Combined effects of Cd and Hg on liver and kidney histology and function in Wistar rats. *J. Agric. Chem. Environ*., **5**, 159–169.

Dorostghoal M., Kazeminejad S.R., Shahbazian N., Pourmehdi M. and Jabbari A. (2017). Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. *Andrologia*, **49**, 1-9.

El S..N. and Karakaya S. (2009). Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. *Nutr. Rev*., **67(11)**, 632-638.

Eidi A., Eidi M. and Darzi R. (2009). Antidiabetic effect of *Olea europaea L*. in normal and diabetic rats. *Phytother. Res*., **23**, 347-350.

El-Demerdash F.M., Yousef M.I., Kedwany F.S. and Baghdadi H.H. (2004). Cadmium induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and beta-carotene. *Food Chem. Toxicol.*, **42(10)**,1563-71.

Ellman G.L. (1959). Tissue sulfhydryl groups. *Arch Biochem. Biophys*., **82**, 70-77.

Ersan Y., Ari I. and Koc E. (2008). Effect of cadmium compounds (cadmium parahydroxybenzoate and cadmium chloride) on the liver of mature mice. *Turk. J. Zool*., **32(2)**, 115–119.

Fernandez-Bolanos J.G., Lopez O., Juan F., Fernandez-Bolanos J. and Rodriguez-Gutierrez G. (2008). Hydroxytyrosol and derivatives: Isolation, synthesis, and biological properties. *Curr. Org. Chem*., **12**, 442-463.

Ferreira I.C.F.R., Barros L., Soares M.E., Bastos M.L. and Pereira J.A. (2007). Antioxidant activity and phenolic contents of *Olea europaea* L. leaves sprayed with different copper formulations. *Food Chem*., **103**, 188–195.

Fotakis G. and Timbrell J.A. (2006). Modulation of cadmium chloride toxicity by Sulphur amino acids in hepatoma cells. *Toxicol. In Vitro*, **20**, 641-648.

Ghorbel I., Elwej A., Fendri N., Mnif H., Jamoussi K., Boudawara T., et al. (2017). Olive oilabrogates acrylamide induced nephrotoxicity by modulating biochemical and histological changes in rats. *Ren. Fail*., **39(1)**, 236-45.

Gong P., Chen F.X., Ma G.F., Feng Y., Zhao Q.Y. and Wang R. (2008). Endomorphin 1 effectively protects cadmium chloride induced hepatic damage in mice. *Toxicol*. **251**, 35–44.

Grosicki A. and Kowalski B. (2002). Whole-body and organ retention of cadmium after repeated administration to rats. *Bull. Vet. Inst. Pulawy*., **46**, 143-147.

Hartwig A. and Schwerdtle T. (2002). Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. *Toxicol. Lett*., **127**, 47-54.

Heim K.E., Tagliaferro A.R. and Bobilya D.J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem*., **13**, 572-584.

Jemai H., El-Feki A, and Sayadi S. (2009). Antidiabetic and Antioxidant Effects of Hydroxytyrosol and Oleuropein from Olive Leaves in Alloxan-Diabetic Rats. J. Agricul. Food Chem., **57(19)**, 8798-804.

Jemai H., Anwar F., Mahmoudi A., El-Feki A., Bouallagui Z. and Sayadi S(2019). Oleuropein protects kidney against oxidative and histopathological damages in subchronic cadmium intoxicated mice**.** *Indian. J. Experim. Biol*., **57(08)**, 602-609.

Julin B., Wolk A., Johansson J.E., Andersson S.O., Andrén O. and Akesson A. (2012). Dietary Cadmium Exposure and Prostate Cancer Incidence : A Population-Based Prospective Cohort Study. *Br. J. Cancer*, **10(5)**, 895–900.

Kamboh A.A. and Zhu W.Y. (2013). Individual and combined effects of genistein and hesperidin supplementation on meat quality in meat-type broiler chickens. *J. Sci. Food Agr*., **93**, 3362-3367.

Liss G., Greenberg R.A. and Tamburro C.H. (1985). Use of serum bile acids in the identification of vinyl chloride hepatotoxicity. *Am. J. Med*., **78**, 68-73.

Lockyer S., Rowland I., Spencer J.P.E. et al. (2017). Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: a randomised controlled trial. *Eur. J. Nutr*., **56**, 1421-1432.

Manach C., Scalbert A., Morand C., Rémésy C. and Jiménez L. (2004). Polyphenols: Food Sources and Bioavailability. *Am. J. Clin. Nutr.*, **79(5)**, 727-47.

Sinha M. and Sil P.C. (2008). Amelioration of cadmium-induced cardiac impairment by

taurine. Chem. Biol. *Interact*., **174(2)**, 88-97.

Mężyńska M. and Brzóska M.M. (2018). Review of polyphenol-rich products as potential protective and therapeutic factors against cadmium hepatotoxicity. *J. Appl. Toxicol*., **39(1)**, 117-145.

Miles E.A., Zoubouli P. and Calder P.C. (2005). Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human with blood cultures. *Nutr*., **21**, 389-94.

Mohamed N.E. (2019). Effect of Aqueous Extract of *Glycyrrhizaglabra* on the Biochemical Changes Induced by Cadmium Chloride in Rats. *Biol. Trace Elem. Res*., **190(1)**, 87-94.

Morin D., Zini R., Tillement S.P. and Burdeau A. (2004). Prevention of cell domageinischemic-reperfusion: mitochondrial respiratory chain as a pharmacological target. *Lett. Drugs Discov*., **1**, 279-284.

Moss D.W., and Butterworth P.J. (1974). Enzymology and medicine. Pitman Med, London 6, 3-11.

Mousa H.M., Ismail M.S., Al-Hassan A.A., Ammar A.S. and Abdel-Salam A.M. (2014). Anti-diabetic effect of olive leaves extract in alloxan-diabetic rats. *J. Agricul. Veter. Sci*., **267**, 1-10.

Nampoothiri L.P. and Gupta S. (2008). Biochemical effects of gestational co-exposure to lead and cadmium on reproductive performance, placenta and ovary. *J. Biochem. Mol. Toxicol*., **22(5)**, 337–344.

Concepción N.M,. Pilar M.M., Martín A., Jiménez J. and Pilar U.M. (1993). Free radical scavenger and antihepatotoxic activity of *bosmarinus tonwntosus*. *Planta Med*., **59**, 312-314.

Nordberg G.F., Nogawa K., Nordberg M. and Friberg L. (2007). Cadmium. Chapter 23. Handbook on the toxicology of metals, 3rd edition, Academic Press, Elsevier, 446-486.

Ognjanovic B.I., Markovic S.D., Pavlovic S.Z., Zikic R., Stajn A.S. and Saicic Z.S. (2008). Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: effect of selenium. *Physiol. Res*. **57**, 403-411.

Oh S.H., Jeong-Eun C. and Sung-chul L. (2006). Protection of Betulin against Cadmium Induced Apoptosis in Hepatoma Cells. *Toxicol*., **220(1)**, 1–12.

Olmo-García L., Bajoub A., Benlamaalam S., Hurtado-Fernández E., Bagur-González M.G., et al. (2018). Establishing the Phenolic Composition of *Olea europaea L*. Leaves from Cultivars Grown in Morocco as a Crucial Step Towards Their Subsequent Exploitation. *Molecules*, **23(10)**, 2524.

Özcan M.M. and Matthäus B. (2017). Benefit and bioactive properties of olive (*Olea europaea* L.) leaves. A review: *Eur. Food Res. Technol*., **243**, 89-99.

Ranieri M., Di Mise A., Difonzo G., Centrone M., Venneri M., Pellegrino T., et al. (2019). Green olive leaf extract (OLE) provides cytoprotection in renal cells exposed to low doses of cadmium. *PLosONE*, **14(3)**, e0214159.

Renugadevi J. and Prabu S.M. (2010). Cadmium induced hepatotoxicity in rats and the protective effect of naringin. *Exp. Toxicol. Pathol*., **62**, 171–181.

Sanjeev S., Bidanchi R.M., Murthy M.K. et al. (2019). Influence of ferulic acid consumption in ameliorating the cadmium-induced liver and renal oxidative damage in rats. *Environ. Sci. Pollut. Res*., **26(20)**, 20631-20653.

Savournin C., Baghdikian B., Elias R., Dargouth-Kesraoui F., Boukef K. and Balansard G. (2001). Rapid high-performance liquid chromatography analysis for the quantitative determination of oleuropein in *Olea europaea* leaves. *J. Agric. Food Chem*., **49(2)**, 618-621.

Seok S.M., Park D.H., Kim Y.C., Moon C.H., Jung Y.S. and Baik E.J. (2006). COX-2 is associated with cadmium-induced ICAM-1 expression in cerebrovascular endothelial cells. *Toxicol. Lett*., **165**, 212-220.

Stohs M,. Shimada A., Zhang B. and Tohyama C. (2000). Renal toxicity caused by cisplatinum in glutathione-depleted metallothionein-null mice. *Biochem. Pharmacol*., **60**,1729-1734.

Swiergosz-Kowalewska R. (2001). Cadmium

distribution and toxicity in tissues of small rodents. *Microscopy Res. Techn*., **55(3)**, 208-22.

Szychlinska M.A., Castrogiovanni P., Trovato F.M. et al. (2019). Physical activity and Mediterranean diet based on olive tree phenolic compounds from two different geographical areas have protective effects on early osteoarthritis, muscle atrophy and hepatic steatosis. *Eur. J. Nutr*., **58(2)**, 565-581.

Wasowicz, W., Gromadzinska, J., and Rydzynski, K. (2001). Blood concentration of essential trace elements and heavy metals in workers exposed to lead and cadmium. *Intern. J. Occup. Med. Environ. Health*, **14**, 223–229.

Wimmer U., Wang Y., Georgiev O. and Schaffner W. (2005). Two major branches of anti-cadmium defense in the mouse: MTF-1/metallothioneins and glutathione. *Nucleic Acids Res*., **33(18)**, 5715-5727.

Zaidi S.M. and Banu N. (2004). Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. *Clin. Chim. Acta*, **340(1)**, 229-233.