Anti -fibrogentic and hepatoprotective potential of methanolic olive extract on cadmium induced toxicity in rats

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Abstract: Cadmium considers one of non-essential toxic heavy metals which affects biological tissues and produces major pathological disorders. Thus, in this study, metanolic extract of olive leaf was analyzed for antioxidant activity, antifibrotic, and hepatoprotective activity against cadmium toxicity inducing liver cell fibrosis in rats. Male wistar rats were classified into four equal groups of ten rats each; group I (control), group II (Cd- treated), group III (OLE + Cd), group IV (vit E + Cd). Cd, OLE, and vitamin E in a doses of 5.6 mg/kg bw and 300 mg/kg bw for both OLE and vit. E were administrated orally for six weeks. Hepatic, oxidative, and fibrogentic biomarkers as well as cadmium traces were estimated in blood and liver tissues following six weeks of treatments. Also, OLE active constituents were estimated using HPLC and colorimetric assays. Compared to Cd- intoxicated rats, hepatic and oxidative biomarkers; AST, ALT, ALP, bilirubin, albumin, MDA, and TAC were significantly improved following administration of OLE and vitamin E in doses of 300 mg/kg bw. In addition, the expression of HYP as measures of fibrogenesis and collagen synthesis showed significant reduction in OLE and vit E treated rats in comparison with Cd-treated rats. The data of HYP were positively correlated with hepatic biomarkers, fibrosis scores, and negatively with MDA and TAC. The antifibrotic and antioxidant property of OLE were significantly related to its phenolic contents especially oleuropein. Cd induces marked oxidative stress and multiple liver cell damage via initiation of oxidative stress and fibrogensis mechanisms and finally liver cell fibrosis. Treatment strategies with olive leaf extracts or vitamin E significantly reduce free radical oxidative stress damage, fibrogenesis of liver cells and finally may help in reversing liver fibrosis. Thus, Olive as dietary supplements could be a promising anti-fibrotic and hepatoprotective agent especially in the highly Cd-polluted environment.
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1. Introduction:

Heavy metals are classified as persistent environmental contaminants present naturally as constituents in the earth. They reached to human and animal bodies through food, air, and water and over accumulated by time. Cadmium considers one of non-essential toxic heavy metals which showed diverse effects depending on the dose. It greatly affects biological tissues and cellular processes through membrane damage, disruption of electron transport, enzyme inhibition and DNA alteration (1-2).

Hepatotoxicity occurs following acute exposure to cadmium appears through different changes particularly swelling of hepatocytes, a wide area of focal necrosis or necrosis, and fatty changes. It was reported that cadmium toxicity performed via promoting the peroxidation chain reaction which results in oxidative stress free radicals within target organs and finally liver proteins and DNA damage with alteration in gene expression (3-6). Also, continuously exposure to environmental heavy metals such as cadmium, leads to initiation of various liver diseases especially cirrhosis and fatty liver (7-9).

Olive plant is recommended as source for many active constituents used as remedy in various diseases (10-13). Different parts of the plant showed vital care activities against many diseases (14-16). Owing to their pharmacological usage, olive leaf and its extracts are shown to be biologically active due to their in vitro antioxidant and antimicrobial activities as well as in vivo activity, by their blood pressure-lowering, hypocholesterolemic, anti-diabetic, anti-inflammatory, antifibrotic, and hepatoprotective effects (17-18).

The bioactivity of olive leaf extract was shown to be associated with the antioxidant, anti- diabetic, antimicrobial of the non-toxic phenolic components particularly oleuropein which constitutes the main phenolic component in olive leaf followed by hydroxytyrosol, oleuropein aglycone, and tyrosol (17, 19-24). It is hypothesized that phenolic compounds present in olive leaf extract, may be able to ameliorate cadmium toxicity inducing liver cell fibrosis. Thus, in this study, methanolic extract of olive leaf was analyzed for antioxidant activity, antifiabrotic, and hepatoprotective activity against cadmium toxicity inducing liver cell fibrosis in rats

2. Materials and methods:

2.1. Materials: Olive leaves were purchased from the local spice shop (Othaim Markets) in Riyadh, Saudi Arabia. All reagents used for the determination of fibrogentic, biochemical, and oxidative indices were purchased from Sigma chemicals (St Louis, Mo, USA).

2.2. Extract preparation: A total of 500 gram of air dried olive leaves was ground with a mortar and pestle under liquid nitrogen and then 10 mL of 80% methanol (80% MeOH) solvent were added. The mixture was allowed to stand in the dark for 24 h. The extract was centrifuged at 5000 g for 10 min, at room temperature, and the supernatants were then filtered using a filter paper and further squeezed to discharge the remaining solutes. All extraction procedures run under dim light to avoid effect of light on olive active constituents.

2.3. Phytochemical Screening of olive leaf extract Various phytochemical screening tests were estimated by using standard procedures for the identification of the phytoconstituents present in methanolic extract of olive leaves (OLE)) (25).

2.4. Assessment of both Total Polyphenolic and Flavonoid Contents. Total polyphenols (TPC) and flavonoid contents (TFC) were estimated in olive extract by using UV-spectrophotometric analysis and both Folin-Ciocalteu (0.5 ml/3ml DW) and aluminum chloride (AlCl3; 0.5mL/2% ETOH ethanol) as working reagents as previously reported (26,27). Standard calibrated curves of gallic acid and quercetin were used to estimate poly phenolic at 650 nm and flavonoids compounds at 420 nm respectively in olive leaf samples. In addition, oleuropein as the main active constituent was estimated in olive leaves extract by using HPLC method. Oleuropein content was estimated in 25 % (w/w) of the OLE product and was kept in the dark at −20° C until studied (28, 29).

2.5. Animals and study design Fifty healthy male wistar rats, weighing between 150 and 200g included in this study. Animals obtained from the authorized experimental animal care center, college of science, King Saud University, Riyadh, Saudi Arabia. They housed in polyethylene cages in groups of 10 rats per cage and kept at a constant temperature (22±2◦C), humidity (55%) and 12 h light–dark conditions. The animals were provided with diet and free access to drinking water. The experiment and the procedures were approved according to Ethics Committee of the Experimental Animal Care Society at King Saud University (Permit Number: PT 1204). Animals with no history of surgery, infection, and other medical interventions, randomly assigned to five groups of 10 each.

2.6. Experimental Design The animals were classified into four groups (10 rats per each group); Group I (Control): Rats received normal saline 2 ml/kg bw, Group II (Cadmium treated): Rats received tenth of cadmium chloride (5.6 mg/kg b.w.) orally through stomach tube day after day for six weeks. Group III (OLE+Cadmium): Animals in this group were given olive leaf extract (OLE: 300mg/kg b.w.) and one hour later, cadmium chloride was administrated (5.6 mg/kg bw). Group IV (Vitamin + Cadmium): Animals in this group were given vitamin E (300mg/kg body weight) and one hour later cadmium chloride was administrated (5.6 mg/kg body weight). Following six weeks of treatment, rats of all groups were sacrificed, dissected, and samples of blood and liver tissue samples were collected for biochemical and histological analysis.

2.7. Acute toxicity test Toxicity studies were conducted as per internationally accepted protocol drawn under OECD guidelines in Wistar albino rats at a dose level of extracts up to 2000 mg/kg b.w. In this study, the toxic effect of the methanolic extracts of olive leaves studied at a dose levels up of 100, 300, and 400 mg/kg b.w in 10 rats. During the period of the experiment, animals examined for any signs of intoxication, lethargy, behavioral modification and morbidity (30-31). In addition, cadmium toxicity in rats was estimated to determine lethal and sub lethal doses LD50. For cadmium chloride, the LD50 was 56 mg/kg b.w.

2.8. Assessment of cadmium in liver tissues Liver tissue homogenates were prepared from one gram of liver tissues digested in 5 ml of mixture of concentrated HNO3 and HCl (1:4 v/v) and left overnight to solubilize the tissue as previously described (32). Following digestion, the mixture was heated at 100^{o} C for 20 minutes in water bath and allowed to cool. Finally, 1.0 ml of hydrogen peroxide was added and the samples were then diluted to a final volume of 25 ml with distilled water before cadmium analyses by Atomic Absorption Spectrophotometer (32).

2.9. Assessment of liver function Following an overnight fast, the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and the concentrations of albumin and bilirubin were estimated in serum samples of all rat groups using commercial kits from Sigma Munich (Munich, Germany) and Boehringer–Mannheim (Mannheim, Germany).

2.10. Assessment of hydroxyproline (HPX) as fibrogenesis markers Liver tissue homogenates of all rats were subjected to estimate hydroxyproline content as previously reported (33). A constant dried tissue samples (at 60°C in a hot air

oven) were hydrolyzed in 6N HCl for 4h at 130°C and then were subjected to Chloramine-T oxidation at pH 7.0 for 20 min. After 5 min, a solution of 0.4M perchloric acid was added to block the reaction. In each sample, Ehrlich reagent was added and the developed color was analyzed at 557 nm in ultraviolet (Systronics-2203) spectrophotometer. A standard curve of the pure L-hydroxyproline was used to calculate the hydroxyproline content in the tissue samples (33).

2.11. Assessment of oxidative biomarkers in liver tissues Spectrophotometer analysis with the aid of colorimetric assay kit (BioVision Incorporated, CA, USA) was used to estimate the concentrations of total antioxidant capacity (TAC) in liver tissues. The results were determined at 570 nm and calculated as a function of Trolox concentration as follows; Sa/Sv = nmol / µl or mM Trolox equivalent, whereas; Sa = Sample amount (in nmol) which has been read from the standard curve, Sv = The undiluted sample volume added to the wells. In addition, MDA was estimated in liver tissues as previously reported (34-35). The tissues were digested with a mixture of TBA (1.5 mL), 8.1% SDS (200 ll), 20% acetic acid (1.5 mL), and distilled water (600 ll). The resultant pink color was measured colorimetrically at 534 nm using a spectrophotometer. MDA concentration per tissue samples was calculated against standard calibration curve as previously described (34-35).

2.12. Statistical analysis The data were statistically analyzed using statistical software, GraphPad, InStat 3. The results are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons were used to study the significance of studied variables. The data were considered significant at $P < 0.05$ levels.

3. Results

3.1. Phytochemical screenings of olive leaf extract (OLE): The methanolic OLE showed 28.6 % w/w of yield. Phytochemical screening analysis reported the presence of flavonoids, glycosides, triterpenoids, carbohydrates, steroids, saponins, polyphenols, and proteins were estimated in OLE (Table1). Considerable amounts of phenolic contents {365.1±15.4 mg of gallic acid equivalents (GAE)/g} and flavonoid {615±21.2 mg of quercetin equivalents (QE)/g} contents were estimated in methnolic OLE extract. Oleuropein as a major phenolic active constituent comprises 28% w/w (128.1±4.2) of the total OLE (Table 1).

3.2. Estimation of cadmium concentration in Cd intoxicated rats treated with OLE and vit E: Cadmium concentration was estimated by using atomic absorption spectroscopy in blood and liver tissues of all groups (figure 1). The data revealed a significant higher traces of cadmium in blood (0.28 µg/g) and liver (0.46 µg/g) of Cd-intoxicated rats compared with lower traces of cadmium estimated in blood (0.018 µg/g) and liver (0.028 µg/g) of OLE treated rats, and vit E treated rats blood (0.026 µg/g) and liver (0.031 µg/g) respectively (figure 1).

3.3. Effect of OLE on pathological and liver biochemical functions: Acute toxicity test of OLE extracts at doses of 100, 300, and 400 mg/kg bw showed no toxicity and lethality (LD50 value=0) at the studied values up to 400mg / kg of the OLE in the animals. Table (2) shows changes in liver weight and liver biochemical functions in Cd rats treated with OLE and vit E. The data showed that liver to body ratio was significantly regulated (improved) in rats treated with OLE and vitamin E compared to Cd-intoxicated rats. In addition, rats treated with OLE and vita. E showed significant improvement in the activities of ALP, ALT, AST, albumin, along with significant reduction in the levels of bilirubin in comparison with Cd-intoxicated rats (Table 2). Cd-toxicity results in an increase in liver fibrosis score (80%) which significantly reduced into 30 % and 50 % in rats treated with OLE and vit E respectively (Table 2).

Table1: Phytoconstituents screening, percentage yield, and quantitative phytochemical contents of olive leaf extarct (OLE mg/ 500mg)

Item	OLE mg/ 500mg
Percentage yield	38.6 %
Phytochemical screening (+/-):	
Alkaloids	-
Flavonoids	+
Tannins	-
Glycosides	+
Triterpenoids	+
Carbohydrates	+
Steroids	+
Saponins	+
Polyphenols	+
Proteins	+
Phytochemical constituents (M ± SD)	
Total polyphenolic content [1]	365.1± 15.4
Total flavonoid content [2]	615± 21.2
Oleuropein %(µmol/g)	28% (128.1± 4.2)

(+/-) presence or absence of phytoconstituents; phytochemical constituents represent as mean ±SD (n = 5). [1] Expressed as mg of gallic acid equivalents (GAE)/g of the dry extract. [2] Expressed as mg of quercetin equivalents (QE)/g of the dry extract.

1.1. Effect of OLE on oxidant-antioxidant status in liver of Cd- intoxicated rats: Figure 2: shows the effect of OLE on oxidative free radicals initiated in relation to Cd- toxicity. Compared to control group, Cd-intoxicated rats showed significant increase in the level of liver MDA and decrease in the activity TAC as a markers of oxidative stress initiated in relation to Cd- toxicity. In addition, when OLE and vit E were administrated for six weeks significant reduction in MDA, and improvement (rise) in TAC activity was reported in rat groups treated with OLE and vit e respectively (Figure 2A, 2B).

1.2. Effect of OLE on hydroxyproline (HPX) expression in liver of Cd- intoxicated rats: Hydroxyproline (HPX) as marker of liver fibrosis was estimated in liver tissue of Cd- intoxicated rats and treated with OLE and vita E (figure 3). In Cd-treated rats, HPX was significantly increased in rats with established fibrosis (F2- F3) compared to those of no fibrosis (F0–F1) and controls (Figure 3A, 3B, 3C). In addition, rats treated with OLE and vit E for six weeks showed significant reduction in HPX expression compared to Cd- treated rats (Figure 3A). More reduction in HPX expression was reported in rats with no fibrosis (F0–F1) compared to those with significant liver fibrosis (F2- F3) as shown in figure (3B, 3C). HPX as a marker of liver fibrosis showed significant correlation with liver cell damage, oxidative stress, and fibrosis scores (Table 3). HPX correlated positively with ALP, ALT, AST, albumin, bilirubin, fibrosis scores, and negatively with oxidative stress markers; MDA and TAC.

Table 2: Regulation of liver-to-body weight ratio and levels of ALT, AST, ALP, albumin, and bilirubin in serum of cadmium intoxicated rats treated with olive leave extract (OLE).

Parameters	CON (n=10)	Cadmium (n=10)	OLE+Cadmium (n=10)	Vitamin E +Cadmium (n=10)
LW/BW (mg/g)	31.2 ±2.5	78.5 ±2.9 [a]	62.5 ±4.1 [b]	73.7 ±1.6 [b]
ALP (U/L)	73.4 ±2.18	125.2±16.7 [a]	61.5±6.3 [b]	65.9±7.5 [b]
ALT (U/L)	26.2 ±3.1	48.1 ± 4.76 [a]	28.9 ± 1.9 [b]	31.4±2.7 [b]
AST (U/L)	39.1±5.4	85.8 ±7.9 [a]	35.2 ±1.5 [b]	38.6±8.1 [b]
Albumin (mg/dl)	5.2±1.3	2.9±0.96 [a]	4.2±1.6 [b]	3.9±0.68 [b]
Bilirubin (mg/dl)	0.86±0.26	6.3±2.6 [a]	2.4±0.96 [b]	3.1±1.21 [b]
Fibrosis score: (N, %)				
No fibrosis (0-1)	-	2 (20 %)	7 (70 %)	5 (50%)
Fibrosis (2-3)	-	8 (80 %)	3 (30 %)	5 (50%)

Data are expressed as the mean ± the standard error of the mean (n = 15),.[a] $p < 0.01$ vs. Control group; [b] $p < 0.001$ vs. cadmium group. ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; LW, liver weight; BW, body weight.

Table (3) Correlation between hepatic hydroxyproline content and different variables among cadmium, olive leave extract, and Vitamin E treated rats.

Variables	Hepatic hydroxyproline levels					
	Cd group		Cd +OLE		Cd + Vit. E	
	r	P	r	P	r	P
ALT (IU/L)	0.251	0.001	0.325	0.001	0.687	0.01
AST (IU/L)	0.365	0.05	0.635	0.01	0.328	0.05
Albumin (mg/dl)	0.214	0.002	0.385	0.01	0.542	0.001
Bilirubin (mg/dl)	0.359	0.01	0.715	0.01	0.368	0.002
MDA	-0.214	0.05	-0.328	-0.001	-0.347	0.002
TAC	-0.325	0.001	-0.125	0.01	-0.378	0.001
Fibrosis score (0-1;2-3)	0.358	0.01	0.264	0.05	0.318	0.001

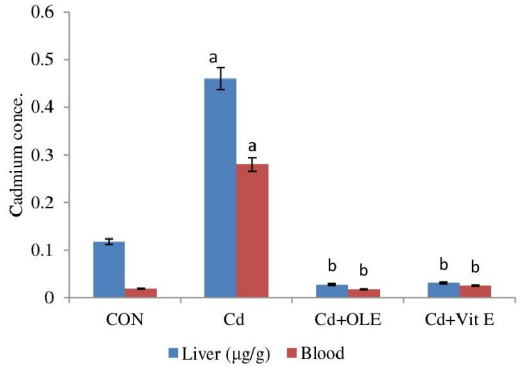

Figure 1: Cadmium concentrations in blood and liver tissue samples of rats intoxicated with $CdCl_2$ (5.6 mg/kg bw), and treated with OLE (300mg/kg) and vit E (300mg/kg). Values are expressed as mean±SD.[a] $p < 0.01$ vs. Control group; [b] $p < 0.001$ vs. cadmium group. CON, control; Cd, cadmium; OLE, olive leaf extract; Vit E, vitamin E.

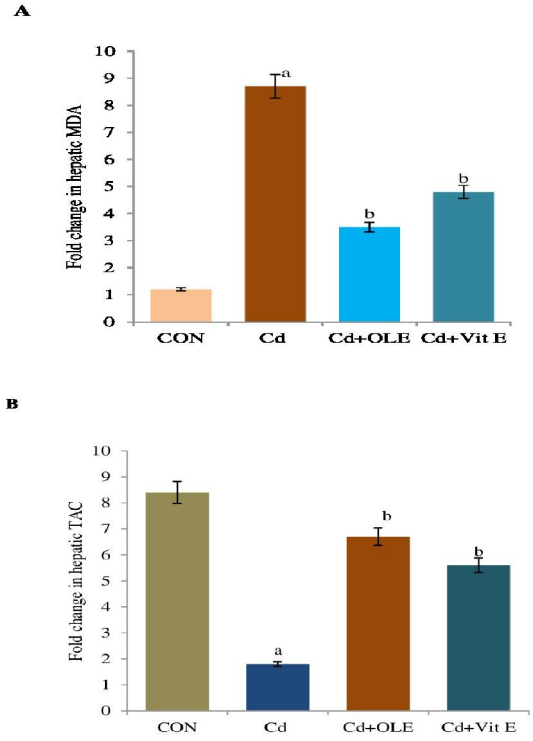

Figure 2: Effect of cadmium toxicity on oxidative stress related biomarkers in liver tissues, and potential effects of olive leaf extracts (OLE). [A] Fold change of hepatic malonyldialdehyde (MDA) as a marker of oxidative stress in liver tissues (nmole / g wet tissue). MDA levels significantly increased in livers of cadmium treated rats compared to both control, OLEE, and Vit.E treated groups. [B] Fold change in total antioxidant capacity (TAC; nmol/mM) in hepatic cells of cadmium treated rats, OLEE, and Vit.E treated groups. Significant increase in TAC activity was reported in OLE and Vit E rats compared to cadmium treated rats. [All values represent mean ± SD.[a] $P < 0.05$; [b]$P < 0.001$; compared to control; Student's t-test.

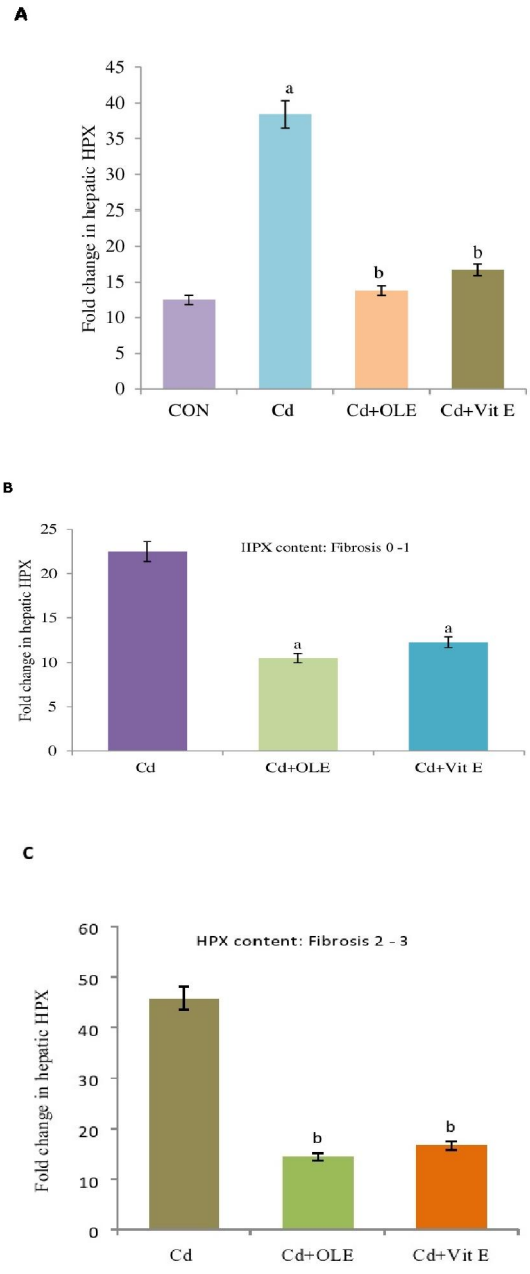

Figure 3: change in hydroxyproline (HPX) expression in liver tissues of [C] control, cadmium, OLE, and vitamin E treated rats; change according to fibrosis score [D] Rats without fibrosis (0-1), and [E] Rats with fibrosis (2-3). hydroxyproline (HPX) as marker of liver cell fibrosis induced by cadmium –toxicity in experimental rats showed significant reduction in expression levels following OLE, and vitamin E treatments for six weeks. All values represent mean ± SD. [a] $P < 0.01$; [b] $P < 0.001$ compared to control. Student's t-test.

2. Discussion

Medicinal plant extracts showed an increasing interest as non-drug remedy for many diseases. This may be due to its flavonoids and other polyphenols constituents that contribute in modulation of many biological process especially in vivo oxidative balances, inflammatory as well as damage of cells and tissues (36-37).

Olive (*Olea europaea* L.) has been traditionally used for centuries to prevent and treat many diseases. The health promoting compounds extracted from olive leaves were applied as potential anti-cancer [38], anti-inflammatory [39], anti-microbial [40], antioxidants (41-42), wound healing (43) or anti-diabetic (44) agents as well as anti-fibrotic, and hepatoprotective effects (17-18).

In this study, phytochemical screening of methanolic OLE showed the presence of flavonoids, glycosides, triterpenoids, carbohydrates, steroids, saponins, polyphenols, and proteins were estimated in OLE. Considerable amounts of polyphenols (365.1 mg) and flavonoid (615 mg) were determined with 500g of OLE. These metabolites originally used by plants for protection against herbivores. Also, it may provide with some pharmacological activity when tested on animals. In addition, Oleuropein as a main active product constitutes up to 28 % of OLE total product as measured by HPLC analysis (28, 29). These products were previously reported to occur in olive leaf (45, 46). The potential antioxidant, anti-diabetic, antimicrobial activities of OLE were shown to be correlated with the presence of the non-toxic phenolic components present in olive leaf, particularly oleuropein followed by hydroxytyrosol, oleuropein aglycone, and tyrosol (47-53).

Previous research studies showed that secondary metabolites commonly synthesized in medicinal plants significantly responsible for various pharmacological properties (54). Flavonoids, glycosides, triterpenoids, carbohydrates, steroids, saponins, polyphenols present in OLE revealed various therapeutic potentials against many diseases (54-56).

Form the previously mentioned various therapeutic potentials of OLE, we tried in this study to evaluate the anti-fibrotic and hepatoprotictive activities of OLE against Cd- induced liver cell damage and fibrosis. Cd- treated rats showed significant increase in the levels of hepatic marker enzymes, there was significant increase in the levels of ALP, AST, ALT, and serum bilirubin as well as a reduction in the levels of albumin. Consistent to our results, other studied showed a change in hepatic serum markers following administration of heavy metals especially cadmium (57-60). The release of hepatic enzymes into the blood circulation results from severe liver cell damage caused by Cd toxicity which in most cases is linked with inflammation, hepatocellular liver injury, destruction of hepatocytes and subsequently an increase in the permeability of the hepatocyte membrane (57,59,60). In addition, peroxidative effect initiated by cadmium was shown to effect on plasma membrane of hepatocytes leading to enzyme leakage into the extracellular fluid (57). Similarly, recent results showed an elevation in the activities of hepatic marker enzymes AST, ALT, and GGT in rats following administration of cadmium (61).

In this study, a significant improvement in the activities of hepatic markers was also estimated when rats treated with methanolic OLE extract and vitamin E respectively for six weeks. There was significant reduction in the activities of ALP, AST, ALT, and serum bilirubin as well as an improving in the levels of albumin in comparison with Cd- intoxicated rats.

Olive leaves were considered as a useful source of highly valuable products (62-63). Oleuropein in glycosylated form was reported as the main natural phenolic antioxidant compound present in high concentration in olive leaves (64), olive oil (65), whereas positive health outcomes were significantly associated with the antioxidant properties of biological constituents occurs in olive oil (66). Consistent to our data, previous results suggested a significant decrease in the activities of hepatic enzymes in OLE treated rats with lower changes in liver histopathological stages and concluded that administration of OLE reduce the incidence of bacterial translocation and liver damage in obstructive jaundiced rats which related to significant increase in bilirubin contents (67). Also, previous results revealed the hepatoprotictive activity of aqueous extract of olive leaves against overdose of paracetamol induced destruction of liver cells in male albino rats (68). The potential antioxidant activity present in OLE was shown to be higher in amounts compared to that occurred in vitamin C and vitamin E, this may be due to synergistic effect proceeds between flavonoids, oleuropeosides and substituted phenols (69). Also, compared to other herbal treated experimental models, our data were in line with others who reported significant reduction in the activities of AST and ALT of Cd- intoxicated rats following treatment with M. oleifera leaf extract and suggested that simultaneous treatment with herbal extracts can improve hepatocellular damage and the liver functions in cadmium chloride-induced rats (70-71).

Also, it was reported that olive (Olea europaea) leaves extract possesses hepatoprotective properties against different liver toxicity modes through inhibiting the physiological and histopathological alterations which may be attributed to its antioxidant activity (72). Thus, we estimated both MDA and TAC as measures of oxidative stress in Cd-intoxicated rats

and to support the potential antioxidant activity of OLE against lipid peroxidation mechanisms. The data of our study showed a significant reduction in the levels of liver MDA and an increase in the activity of TAC following administration of OLE and vitamin E respectively. Supporting to our results, other studies showed OLE either alone or in combinations with other herbal constituents such as rosemary leaves showed significant hepatoprotective effects against hepatotoxicants, and suggested antioxidant pathways as modes of potential activity of OLE against liver cell toxicity (72-74). The potential antioxidant activity of OLE was shown to be related to antioxidant properties of oleuropein as the main active constituents. It was shown to regulate or reduce the increased levels of ALT and AST, and oxidative stress paramters in Sprague–Dawley male rats treated with ethanol. They suggested that oleuropein has antiperoxidative effects against ethanol-induced liver toxicity (75).

In a similar fashion, vita E reported a significant reduction in the levels of hepatic biomarkers ALT, ALP, AST, and MDA, TAC compared to Cd-intoxicated rats. Our data are in accordance with others who showed that co-administration of either α-lip or vit E daily for three weeks significantly modulate hepatic and oxidative biomarkers, and proved that these combinations may have a hepatoprotective activity against zinc oxide nano-particles (ZnO-NPs) induced liver cell toxicity (76).

In this study, the accumulation of cadmium in blood and liver tissues was estimated using atomic absorption spectroscopy. The data showed significant increase in the levels Cd traces in blood and liver tissues of rats intoxicated with $CdCl_2$ at doses of (5.6 mg/kg bw) for six week.

Previous research studies reported that Cd rapidly absorbed, transported and binds to essential proteins such as metallothionein and other peptides in the liver tissues (77), and the excess of Cd accumulates in all biological tissues especially the blood cells, kidney, liver (78), which in turn initiates for many adverse effects such as metabolic disorders, membrane cell damage, alter gene function, and promotes gene mutation and apoptosis (79). It was reported previously that both the liver and kidney are the main target organs for progressive accumulation of Cd (80-81).

In addition, lower traces of cadmium were reported in Cd-intoxicated rats following treatment with OLE (300 mg lkg bw), and Vitamin E (300 mg/kg bw) respectively. This may be related to chelating action property of bioactive constituent's present in OLE and vitamin E, the data are in line with those who reported that administration of salinomycinic acid (Sal) as chelating agent significantly decreased the concentrations of the toxic metal ion in liver cells and confirm its promising as antidote to Cd poisoning (82). Also, it was reported that anti-free radical activity of oleuropein proceeds through chelating ions of heavy metals such as Cu and Fe, which catalyze free radical generation reactions (83), and subsequently effects on accumulation of Cd within biological tissues. Conversely to our results, other studies showed that protection of herbal medicine such as curcumin based up on antioxidative mechanism rather than any change reported in tissue Cd concentration (84). Also, it was reported previously that dietary flavonoids provide protection against the toxic effects via induction of cellular Phase II detoxifying enzymes (85).

To study the antibiotic activity of OLE, hydroxyproline (HYP) was estimated in Cd-intoxicated rats. There was significant increase in the levels of HYP produced in Cd- treated rats compared to controls. Also, HYP significantly reduced in Cd-intoxicated rats following oral administration of OLE and vitamin E in dose of (300 mg /kg bw) for six weeks respectively.

The expression of HYP showed significant correlation with hepatic and oxidative metabolic signs as well as fibrosis scores in Cd, OLE, and vitamin E treated rats. HYP correlated positively with ALP, ALT, AST, albumin, bilirubin, fibrosis scores, and negatively with oxidative stress markers; MDA and TAC. Previously it was reported that rats with the metabolic syndrome showed improved or normalized hepatic signs following administration of OLE. These results strongly reveal that OLE bioactive constituents such as oleuropein and hydroxytyrosol reverse fibrotic and collagen deposition via anti-inflammatory and antioxidant mechanisms (86-87).

Other proposed mechanism that OLE and vitamin E and its phenolic counterparts may reduce liver fibrotic process via anti-apoptotic mechanism. OLE and its constituents were shown to reduce the expression of proapoptotic protein Bax and inducing the expression of the expression of antiapoptotic protein Bcl-2, thus shifting the mechanism towards the suppression of apoptosis induced following Cd-toxicity (88-90).

In conclusion, Cd induces marked oxidative stress and multiple liver cell damage via initiation of oxidative stress and fibrogensis mechanisms and finally liver cell fibrosis. Treatment strategies with olive leaf extracts or vitamin E significantly reduce free radical oxidative stress damage, fibrogenesis of liver cells and finally may help in reversing liver fibrosis. Thus, Olive as dietary supplements could be a promising anti-fibrotic and hepatoprotective agent especially in the highly Cd-polluted environment.

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