

# Cytoprotectant and Anti-Oxidant Effects of Olive Oil on Cadmium Induced Nephrotoxicity in Mice

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# Abstract

Background: Cadmium is a heavy metal having toxic effects on most organ systems of the body. *Objectives*: We aim to investigate the postulated protective properties of olive oil in Cadmium-induced renal toxicity in mice by utilizing standard biomarkers of renal toxicity, oxidative stress and histopathological characterization. *Materials and Methods*: Forty mice were randomly divided into four groups of 10 mice each. Group 1 served as control group, group 2 was given Extra-virgin olive oil orally, group 3 was given Cadmium chloride and group 4 was given Cadmium chloride with Extra-virgin olive oil. At the end of the experimental period, biochemical analysis histopathology of kidney was done. *Results*: The present study depicted that blood urea, serum creatinine and the antioxidant markers (Superoxide dismutase, Glutathione peroxidase and Catalase) levels were significantly increased in group 3 compared to group 1 and 2. After administration of Extra-virgin olive oil with Cadmium in group 4, the levels of those markers significantly improved. Histopathology of the renal tissue showed severe damage of glomeruli, severely congested blood vessels and marked dilatation of Bowman's capsule in group 3 with improvements of these changes in group 4. Conclusion: This study suggests that Extra-virgin olive oil can be used as a cost effective safe anti-oxidative agent in the prevention of Cadmium toxicity.

# **Keywords**

Reactive Oxygen Species, Cadmium, EVOO, Nephrotoxicity, Oxidative Stress

## **1. Introduction**

Cadmium (Cd) is a heavy metal having toxic effects on most organ systems of the body [1]. In fact, this environmental pollutant is ranked among the top toxic substances [2]. Human exposure to cadmium occurs through metal industries, contaminated food, air pollution and cigarette smoking [3] [4] [5].

In 1817, it was first proposed by Friedrich Stromeyer (Göttingen, Germany) that cadmium intoxication can cause damage to kidney, bone, and lungs [6]. Accumulation of cadmium occurs mainly in the kidney leading to nephrotoxicity. Cadmium circulates in the blood either in the free form or bound to various carrier proteins such as albumin, glutathione and metallothioneins (MTs) [7]. Free form of cadmium causes the formation of reactive oxygen species (ROS) in the renal cells and induces apoptosis [8]. Renal toxicity primarily damages the initial parts of the proximal tubules especially the S1 and S2 segments [9] [10]. Damage to the proximal tubules results in glucosuria, proteinuria and aminoaciduria.

After filtration from the glomerulus, free form is transported by zinc transporter and divalent cation transporter 1 (DCT1) [11] [12] [13] [14]. Some of the free form is taken care of by other cation transporters like that for calcium and ferrous ions [15]. The bound forms of cadmium utilize the receptor-mediated endocytosis pathway [16] and amino acid transporters [17] for moving across the apical membrane. Zalups proposed that the principal pathway for cadmium entrance into the renal tubular cells may be through the organic cation transporters (OCTs) in the basolateral membrane tubular cells [13].

The cytotoxicity of cadmium results largely from its free form as has been evidenced in many *in vitro* studies [18] [19] [20] [21]. Quamme [22] in his study showed that in the renal MDCK epithelial cells, the free cadmium is transported into the intracellular space across the membranes, which support the probability of accessibility of CdZ+ for the intracellular milieu and the ability to react with sensitive sites in the renal tissues. Furthermore, Goyer and his co-workers [23] demonstrated in their study that nephrotoxicity might be induced by divalent CdZr, and that basolateral Cd uptake may significantly contribute to the total renal burden of Cd under certain conditions [24].

In the diet of Mediterranean area, Olive oil is considered the fundamental source of dietary fat, and different useful effects on the human body health are resulted from its steady consumption [25]. Extra virgin olive oil is well recognized for its antioxidant properties, hypotensive, hypoglycaemic, cardiovascular and hepatoprotective effects [26]. It is also known for its anti-microbial and anti-inflammatory properties [27]. The monounsaturated fatty acids (MUFA) especially oleic acid, which constitutes 70% - 80% of total fatty acids, may be instrumental in the favourable effects of olive oil on the cardiovascular system [28]. Phenols present in the olive oil are potent antioxidants and scavenge free radicals and inhibit low-density lipoprotein (LDL) oxidation [29]. Olive oil contains a wide variety of antioxidants such as vitamin E, oleocanthal, carotenoids and especially oleuropein which prevents the oxidation of LDL particles [30].

One of the studies done by Mohammed *et al.* (2014) concluded that administration of virgin olive oils and nigella sativa improved the abnormalities in the oxidative antioxidant parameters and the altered biochemical markers and ameliorated organ damage caused by cadmium toxicity in rats [31]. Amamou *et al.* (2015) in their study found the protective effect of Olive oil or colocynth oil consumption on the rat liver against Cadmium-induced injury and attributed the effects due to the reduced oxidative stress and the enhancements of antioxidant enzymes activities [32].

The experimental demonstration of the protective activities of olive oil against cadmium-induced nephrotoxicity is lacking. This study aims to investigate the postulated protective properties of olive oil in Cadmium-induced renal toxicity in mice by utilizing standard biomarkers of renal toxicity, oxidative stress and histopathological characterization.

## 2. Materials and Methods

#### 2.1. Mice

We used Male Swiss albino mice, of almost the same age (~2 weeks) and weight (20 - 30 gm) in our experiment. The mice were housed in plastic cages, fed a standard laboratory diet and water *Ad libitum* exposed to a 12 h light/dark cycle, and maintained at a laboratory temperature of  $20^{\circ}$ C ±  $2^{\circ}$ C. The animals were quarantined for 7 days before beginning the experiments. All mice were handled in accordance with the standard guide for the care and use of laboratory animals and according to the guidelines laid by the Ethics committee of Aljouf University.

## 2.2. Chemicals

Cadmium in the form of CdCl<sub>2</sub> was purchased from Sigma-Aldrich (St. Louis, MO, USA). Extra virgin olive oil (EVOO) was purchased from the local market in Sakaka city, Aljouf, KSA. Urea and creatinine kits were purchased from Biovision, USA. The other chemicals were purchased from Sigma (St. Louis, MO, USA).

# 2.3. Experimental Design

Forty mice with average weight of 20 - 30 g at the start of study were used in this experiment. The study was conducted in the Department of Pathology, College of Medicine, Aljouf University. Mice were randomly divided into four groups with 10 rats in each group.

*Group* 1: Animals were given distilled water orally at a daily dose of 2 mL/kg body weight for 28 days, and served as the control group.

*Group* 2: Animals were given extra virgin olive oil orally at a daily dose of 2 mL/kg body weight for 28 days [33].

Group 3: Animals were given 1.8 mg CdCl<sub>2</sub>/kg of body weight once a day

(corresponding to 1/50 LD50), dissolved in saline for a period of 28 days. The average oral lethal dose (LD50) value for  $CdCl_2$  in mice has been reported as 88 mg Cd/kg of body weight [34].

*Group* 4: Animals were given  $CdCl_2$  orally at a daily dose of 1.8 mg  $CdCl_2/kg$  b.w/day and extra virgin olive oil at a daily dose of 2 mL/kg body weight for 28 days.

#### 2.4. Sample Preparation

Mice were fasted for 12 hours overnight at the end of the study period, and all the mice were sacrificed by cervical dislocation under the effect of anaesthesia of diethyl ether. The samples of blood were gathered from the mice and stored until biochemical analysis at  $-70^{\circ}$ C. Ice cold saline buffer (20 mM Tris-HCl, 0.14 M NaCl buffer, pH 7.4) was used to rinse the kidney after its removal and weighing, and then a Potter Elvehjem homogenizer was used to homogenize it in the same minced solution. Lipid peroxidation assay was done immediately using the homogenate tissue, and homogenate aliquots were kept for further biochemical analysis.

#### 2.5. Biochemical Assays

#### 2.5.1. Blood Creatinine and Urea Levels Estimation

Trinder (1969) [35] method was used for assaying *creatinine and urea levels* utilizing the commercial diagnostic kits.

#### 2.5.2. Measurement of Renal Oxidative Markers

The method of Aebi (1984) [36] was used to determine the renal catalase (CAT) activity in tissue homogenates. Renal homogenate activity of superoxide dismutase (SOD) was estimated according to Spitz and Oberley (2001) [37] technique. Renal level of Glutathione peroxidase (GPx) was estimated according to Lawrence and Burk (1976] [38] technique. According to the method of Ohkawa *et al.* (1979) [39] and Yagi (1984, 1976) [40] [41], thiobarbituric acid reactive substances (TBARS) measurement was used to estimate the lipid peroxidation and they was expressed in terms of malondialdehyde content.

#### 2.6. Histopathological Examination

Mice kidneys were immediately immersed in 10% neutral buffered formalin solution and kept for 24 hours followed by 70% ethanol wash. Then tissue pieces were dehydrated by using ascending grades of alcohol and finally paraffin embedding was done. Sectioning was done using a rotary ultra-microtome, and sections were dried overnight. Finally, sections were stained with haematoxylin and eosin (H & E) dyes and slides were observed under a light microscope. All histological slides were analysed by Leica micro imaging systems DMD 4000, USA.

#### 2.7. Statistical Analysis

The collected data from the experiment were presented as mean  $\pm$  SD value. The

table or figure legends have been used to state the number of animals per each group. The mean differences between the studied groups for each parameter were separately analysed using one way analysis of variance (ANOVA) followed by Student-Newman-Keuls test and this is after ascertaining the homogeneity of variance between treatment groups by Bartlett's test. Values of  $p \le 0.05$  were considered of statistical significance.

#### 3. Results

# 3.1. Effect of Cadmium (Cd), Extra Virgin Olive Oil (EVOO) on Body and Kidney Weights

**Table 1** depicts that after 4 weeks breeding of the four groups with matched initial body weight, both final and relative body weights (RBW) were significantly decreased in G3 (the group administered Cd) compared to G1and G2 (control group and group given Extra virgin olive oil, respectively), ( $p \le 0.05$ ). After administration of the protective agents with Cd in G4 (Cd + Extra virgin olive oil), both final and RBW significantly increased compared to G3. Kidney weight and relative kidney weight (RKW) were significantly decreased in G3 compared to G1 and G2 ( $p \le 0.05$ ). The protective agents significantly increased kidney weight and RKW in G4 compared to G3 ( $p \le 0.05$ ) (Table 1).

# 3.2. Effect of Cadmium (Cd), Extra Virgin Olive Oil (EVOO) on Renal Function Tests

**Table 2** showed that blood urea and serum creatinine levels have significantly increased in G3 compared to G1 and G2, ( $p \le 0.05$ ). After administration of EVOO with Cd in group 4, the levels of those markers significantly improved compared to Cd group (G3) ( $p \le 0.05$ ).

# 3.3. Effect of Cadmium (Cd), Extra Virgin Olive Oil (EVOO) on Kidney Oxidative Markers

Table 3 showed that the antioxidant markers (e.g. CAT, SOD and GPx) levels

 Table 1. Effects of cadmium (Cd), extra-virgin olive oil (EVOO) and their combination, on body weight gain, kidney weights of mice groups, after four weeks of treatment.

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	Parameters	G1 (Control)	G2 (EVOO)	G3 (Cd)	G4 (Cd + EVOO)
	IBW (g)	27.33 ± 3.20	$27.66 \pm 3.14$	$27.50\pm3.45$	28.00 ± 2.76
	FBW (g)	$32.16 \pm 1.17$	$32.00 \pm 1.41$	$25.66\pm1.86^{ab}$	$32.00\pm1.67^{\circ}$
	RBW	$118.62\pm9.74$	$116.67 \pm 11.69$	$94.02 \pm 7.83^{ab}$	$114.91 \pm 9.01^{\circ}$
	KW	$2.25\pm0.19$	$2.28 \pm 0.15$	$1.36\pm0.18^{ab}$	$2.08 \pm 0.12^{\circ}$
	RKW	$6.99\pm0.54$	$7.14 \pm 0.50$	$5.37 \pm 1.01^{ab}$	$6.53 \pm 0.63^{\circ}$

Data are mean  $\pm$  SD. G1: control; G2: EVOO; G3: Cd; G4: Cd + EVOO. IBW: Initial body weight; FBW: Final body weight; RBW: Relative body weight; RKW: Relative kidney weight. Relative body weight = (Final body weight/Initial body weight) × 100. Relative kidney weight = (kidney weight/Final body weight) × 100. a. compared to the control (G1) group; b. compared to EVOO (G2) group; c. compared to Cd (G3) group-significant at  $p \leq 0.05$ .

Table 2. Effects of cadmium (Cd), extra virgin olive oil (EVOO) and their combination	ı,
on kidney functions after four weeks of treatment.	

Parameters	G1 (Control)	G2 (EVOO)	G3 (Cd)	G4 (Cd + EVOO)
Bl Urea (mg/dl)	$26.72 \pm 1.88$	$27.90\pm0.84$	$44.60\pm0.83^{ab}$	$30.73\pm0.78^{\circ}$
S. Creatinine (mg/dl)	$0.74\pm0.03$	$0.74\pm0.03$	$0.96\pm0.02^{ab}$	$0.79\pm0.03^{\circ}$

Data are mean  $\pm$  SD. G1: control; G2: EVOO; G3: Cd; G4: Cd + EVOO. a. compared to the control (G1) group; b. compared to EVOO (G2) group; c. compared to Cd (G3) group-significant at  $p \le 0.05$ .

**Table 3.** Effects of cadmium (Cd), extra virgin olive oil (EVOO) and their combination, on oxidative stress markers in the kidneys of mice groups, after four weeks of treatment.

Parameters	G1 (Control)	G2(EVOO)	G3 (Cd)	G4 (Cd + EVOO)
CAT	$290.83 \pm 7.78$	290.33 ± 12.80	$205.00 \pm 11.17^{ab}$	$259.66 \pm 24.40^{abc}$
SOD	45.50 ± 2.94	44.16 ± 2.14	$34.00\pm2.76^{ab}$	$40.66\pm1.96^{abc}$
GPx	$2.48\pm0.22$	$2.46\pm0.24$	$1.35\pm0.10^{ab}$	$2.05\pm0.23^{abc}$
MDA	23.83 ± 1.94	23.33 ± 2.16	$43.66 \pm 3.20^{ab}$	$30.16 \pm 2.32^{abc}$

Data are mean  $\pm$  SD. G1: control; G2: EVOO; G3: Cd; G4: Cd + EVOO. CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde. a. compared to the control (G1) group; b. compared to EVOO (G2) group; c. compared to Cd (G3) group-significant at  $p \le 0.05$ .

have significantly decreased in G3 compared to G1 and G2, while MDA has significantly increased compared to those groups ( $p \le 0.05$ ). After administration of EVOO with Cd in group 4, the levels of those markers significantly improved compared to Cd group (G3) ( $p \le 0.05$ ), however they do not reach the normal levels of the control groups (G1 and G2).

## 3.4. Histopathological Findings

Histopathological examination of the renal tissue from control group (Figure 1) and the olive oil group (Figure 2) showed normal renal architecture whereas Cadmium group showed severe congestion of the renal blood vessels and severe damage of glomeruli, inflammatory cell infiltrate, and widening of glomerular space (Figure 3(a) and Figure 3(b)). EVOO + Cd group depicted nearly normal renal architecture with little inflammatory cell infiltration (Figure 4).

# 4. Discussion

Cadmium (Cd) is one of the common toxic agents that belongs to the heavy metal group. In the humans body, respiratory tract irritation and pulmonary oedema might occur after inhalation of Cd, while bone fractures, renal dysfunction, osteoporosis and anaemia often occurred after chronic Cd exposure [42].

The molecular mechanism accountable for the toxic effects of Cd has not so far been entirely elucidated but it has been proposed that the formation of reactive oxygen species (ROS) leads to oxidative damage in various tissues with consequent loss of function of the membrane [43].



**Figure 1.** Photomicrograph of renal tissue from the Group-I (control group) shows normal renal architecture (H & E ×100).



**Figure 2.** Photomicrograph of renal tissue from the Group-II (olive oil group) shows normal renal architecture (H & E  $\times$ 100).

Long-term exposure to Cd causes derangement of antioxidant system with inhibition of antioxidant enzymes. Many studies proved that the oxidative stress was the major instigator of the toxic effects of cadmium in the kidneys [44]. In this study, we tested the toxic effects of cadmium on kidney. Furthermore, we evaluated the role of EVOO as an antioxidant to recover the tissues from the toxic effects of cadmium.



**Figure 3.** (a) Photomicrograph of renal tissue from the Group-III (cadmium group) shows severe congestion of blood vessels along with edema and inflammatory cell infiltrate. The surrounding glomeruli and tubules are damaged (H & E ×100); (b) Photomicrograph of renal tissue from the Group-III (Cadmium group) shows mononuclear inflammatory cell infiltration, severe damage to some of the glomeruli and widening of the Bowman's space (H & E ×100).

By the end of our experiment, the body weight of the animals was determined in grams and it was found that the control group mice (G1) gained some body



**Figure 4.** Photomicrograph of renal tissue from the Group-IV (Cd + EVOO group) shows near normal renal architecture with few inflammatory cells (H &  $E \times 100$ ).

weight during the experiment referenced to their weight at the start of the experiment. The cadmium alone (G3) group caused the biggest weight loss. Adding EVOO significantly reduced the loss of body weight caused by cadmium. These finding are in agreement with Ibraheem *et al.* (2016) [45] who found that Cd administration to a group of mice caused weight loss. Cadmium binds to sulfhydryl groups and leads to structural distortion of proteins [46]. The ability of EVOO to reduce cadmium effect on body weight is reasonable since EVOO contains many components that have been shown to be fundamental for more than hundreds of the enzymatic reactions and also fundamental for the function and structure of a great proportion of macromolecules [47] [48].

Creatinine is produced in the kidney, liver and pancreas where it is phosphorylated and transported to the brain and muscle tissue [49]. This study demonstrated that serum creatinine and urea were significantly increased in Cd treated group than in control group. These findings are similar to the findings of many researchers [50] [51] [52] whose results depicted the effect of Cd intoxication on serum urea and creatinine levels, where Cd administration caused a significant serum elevation of the levels of the creatinine and urea when compared with the control ( $p \le 0.05$ ). Co-administration with EVOO restores the renal functions to near normal range. This might be caused by the antioxidant effect of EVOO on the Cd toxicity.

To be used as an indicator of lipid peroxidation, the tissue level of MDA was measured in the current study. The kidney tissue level of MDA was significantly elevated in the mice treated and intoxicated with Cd alone. The data available from our study depicted that oxidative stress has an essential role that explained the toxicity and the damage that occur in the kidney tissue. Our findings are in agreement with the previous researches that reported the important role of oxidative stress in Cd toxicity [53] [54] [55]. The increased MDA levels in renal tissue is an indication of the increased levels of lipid peroxides in kidney tissue, causing increased consumption of functional thiol (-SH) groups and its depletion in many antioxidant enzymes, as we noticed in the decreased tissue levels of GPx, SOD and CAT enzymes in the current study. This is in agreement with Liu *et al.* (2008) [56] who stated that DNA damage occurred in Cd induced toxicity as a result from the increased and accumulation of ROS which generate lipid peroxidation. This is in contrast with finding of Thijssen *et al.* (2007) [57] who stated that following oral administration of Cd in chronic studies and after long period of Cd exposure, these is lack of ROS production in the tissues.

Previous studies [58] [59] [60] [61] depict that Cd toxicity is mediated through the reactive oxygen species (ROS), either in intact mice through all exposure routes or, in an assortment of cell culture systems. The mechanism of membranes, proteins and DNA damage that occur in Cd toxicity results from the oxidative stress that encompassed of the imbalance between production and elimination of ROS in the components of tissues and cells [53]. The lipid peroxidation of the membrane-bound polyunsaturated fatty acids in Cd induced toxicity was initiated by the production of ROS, leading to ailment of the functional and structural integrity of the membrane, which is the outcome of an interaction between unsaturated fatty acids and free radicals of diverse origins that are model in the lipids of membrane. Cadmium toxicity induced the polyunsaturated fatty acids degradation in the cell membrane by the generation of ROS, which results in the impairment of membranes and the generation of thiobarbituric acid reactive species, MDA , used as lipid peroxidation indicators with the conjugated dienes [62] [63].

Liu *et al.* (2008) [56] proposed that the glutathione depletion and sulfhydryl group binding properties of cadmium increased the production of ROS and suggested these to be the mechanism responsible for Cd toxicity. The co-administration of EVOO with Cd ameliorated the toxic effect of Cd and restored the antioxidant enzyme levels to their normal state with reduced MDA level.

Administration of cadmium caused severe damage to the renal glomeruli manifesting with damage to the glomerular epithelium and tubules, inflammatory cell infiltrate and severe congestion of the renal blood vessels (**Figure 3(a)** and **Figure 3(b)**). The glomeruli and proximal tubules in the kidney, both of them showed swelling; hyperplasia of the tubules, widening of Bowman's space, interstitial necrosis and fibrosis, atrophy of glomeruli were also observed. Our results were in agreement with those of E. Mohammad [31]; Damek-Poprawa (2003) [62] and Sawicka-Kapusta; Järup (2002) [63] and Ohta (2000) [64].

The administration of olive oil with Cadmium prevented the damage in the kidneys as evidenced by changes in only few glomerular structures and little inflammatory cell infiltrate (**Figure 4**). These changes were observed by Mohammad (2014) [31] who attributed these to the increased activity of the anti-oxidant enzymes and reduced MDA levels in the renal tissues. Olive oil inhibits the lipid and protein peroxidation and improves the antioxidant mechanism because of their high phenolic compounds as has been proved previously [65] [66] [67]. Besides phenolic compounds, other compounds which have beneficial effects include monounsaturated fatty acids, oleic acid and tocopherols [68] [69].

Nakbi *et al.* (2010) [47] reported that the nutrigenomic effects of the polyphenol-rich watery (hydrophilic) components on the body's own antioxidant network are mainly associated with the protective effects of olive oil against oxidative damage. The polyphenol-rich hydrophilic fraction of olive oil appears to be effective in decreasing oxidative stress induced by toxins.

## **5.** Conclusion

The kidney is considered as one of the major target organs affected by Cadmium toxicity. Cadmium can accumulate in the kidneys and cause severe tissue damage, as was observed from the results in our study. Our results demonstrated that Cadmium increases the oxidative stress by depleting CAT and inhibiting the activities of antioxidant enzymes. The treatment with EVOO significantly protected the Cadmium-induced oxidative stress. The study suggests that EVOO can be used as a cost effective safe anti-oxidative agent in the prevention of Cadmium toxicity.

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## **Conflict of Interest Statement**

The authors declare that there are no conflicts of interest.

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# **Abbreviations**

Cadmium: Cd Extra virgin olive oil: EVOO Catalase: CAT Malondialdehyde: MDA Superoxide dismutase: SOD Glutathione peroxidase: GPx Reactive oxygen species: ROS