



Biochemical study on the impact of *Nigella sativa* and virgin olive oils on cadmium-induced nephrotoxicity and neurotoxicity in rats

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ABSTRACT

Objective: Cadmium is an environmental pollutant that ranked as one of the top toxic substances rampant in many industries such as cement plants and smoke, batteries and others. The present investigation aimed to assess the possible protective effects of *Nigella sativa* oil (NSO) and Virgin Olive oil (VOO) on rat administered cadmium chloride (CdCl₂). **Design:** 40 male rats were randomly divided into four groups; 10 rats for each: control group (0.5 ml phosphate-buffered physiological saline orally); Cd-treated group (5mg/kg body weight CdCl₂ orally); NSO + Cd-treated group (1 ml/kg body weight of NSO + 5mg/kg body weight CdCl₂ orally); VOO + Cd-treated group (0.5 ml/kg body weight of VOO + 5mg/kg body weight of CdCl₂ orally). All treatments were administered daily for a period of 4 successive weeks. **Materials and Methods:** Blood and tissue sample were collected at the end of the experimental period. In all groups, potential injuries in brain and kidney were evaluated by using biochemical parameters and histopathological investigations. **Results:** The current study revealed that the administration of CdCl₂ induced significant increase in serum creatinine and urea. It also induced significant increase in CAT, SOD, MDA and DNA fragmentation % in brain and kidneys of rats. In addition serum acetyl cholinesterase activity and tissue GSH content were significantly decreased. The biochemical results were confirmed by histopathological investigations where many lesions were detected in brain and kidneys treated with cadmium. Administration of each of NSO and VOO with CdCl₂ showed a significant improvement in the measured biochemical parameters and histopathological pictures. **Conclusion:** The present study concluded that the VOO and NSO can be used as potentially effective therapeutic agents for workers in factories that their products are contaminated with cadmium particularly cement factories.

KEY WORDS: Antioxidants, cadmium, *Nigella sativa*, olive oil, rats

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INTRODUCTION

Contamination of the environment by several pollutants is one of the most significant problems in this century. As a result of industrial activities, lots of chemical substances such as heavy metals have generated pollution in the environment. Although, some metals such as manganese, zinc, iron, cobalt, and nickel are essential for the metabolism in trace amounts, whereas other nonessentials have no biological role (e.g., silver, aluminum, cadmium, lead, mercury) and potentially toxic for living systems and they can be carried through soil–plant–animal–human cycles [1]. Among these metals due to acute toxicity, cadmium has been considered as an extremely significant pollutant with lead and mercury [2]. Of concern is the increasing concentration of cadmium deposited in agricultural soils by airborne cadmium particles. Cadmium is a

relatively mobile metal in soils, and many crops retain relatively high cadmium levels [3].

Cadmium is an environmental pollutant that ranked as one of the top toxic substances [4]. Food is the major source of cadmium exposure for the general population, and cigarette smoking significantly adds to the body burden of cadmium [5]. Occupational exposures come primarily from cadmium fume inhalation, cadmium-nickel battery industry, electroplating, and paint pigments [4]. Acute cadmium poisoning causes pulmonary edema, hemorrhage, fulminate hepatitis, testicular injury, and lethality; whereas chronic exposure to cadmium produces nephrotoxicity, osteotoxicity, immunotoxicity [5] and neurotoxicity [6]. Cadmium is also classified as a human carcinogen causing tumors of the lung, prostate and other tissues [7]. At the cellular level, exposure to

cadmium induces genomic instability, interaction with DNA repair mechanism, generation of reactive oxygen species and induction of apoptosis [8].

More attentions have been paid to the natural antioxidants owing to its protective effects against heavy metals-induced toxicities, especially whenever reactive oxygen species are involved. Among these natural antioxidants is *Nigella sativa* (NS), which is a member of Ranunculaceae family that grows spontaneously and widely in several Southern Mediterranean and Middle Eastern countries. NS seed has over 100 different chemical constituents, including abundant sources of all the essential fatty acids. Although it is the oil that most often used medicinally, the seeds are a bit spicy and are often used whole in cooking curries, pastries and Mediterranean cheeses [9].

Olive oil is an integral ingredient within the Mediterranean food. There is growing evidence that it may have great health benefits including the prevention of coronary heart disease risk, some cancers and the modification of immune and inflammatory responses [10]. Virgin olive oil (VOO) is a functional food with various components such as monounsaturated fatty acids (MUFA) that may have nutritional benefits. It is also a good source of phytochemicals, including polyphenolic compounds [11]. Based on these facts our study was designed to investigate the toxic effects of cadmium chloride (CdCl_2) on brain and kidneys of rats and to study the protective effects of *Nigella sativa* oil (NSO) and VOO on CdCl_2 -induced toxicity in rats.

MATERIALS AND METHODS

Animals and Chemicals

This study was conducted on 40 adult male Wistar rats of an average body weight 120-150 g. They were obtained from Helwan farm of laboratory animals Cairo; Egypt. Rats were kept at optimum temperature ($24 \pm 2^\circ\text{C}$) and humidity (55-60%) under 12:12 h light-dark cycle. After acclimatization, rats were divided into 4 groups ($n = 10$). Rats were given uniformly basal diet and water ad libitum. CdCl_2 (99.0% purity) was purchased from Loba Chemie Company (India). NSO was purchased from Cap Pharm for extracting natural oils, Egypt. Extra VOO was obtained from Sinai, Egypt. Its fatty acids composition is: 75% monounsaturates, 13% polyunsaturates and 12% saturates, and phenolic concentration is 200 mg/100 g olive oil. All measured reagents were purchased from Sigma Aldrich Co., USA.

Experimental Design

A total of 40 male albino rats weighs $120-150 \pm 10$ g were used. The animals were divided into 4 groups (10 per each) and were treated as follows for a period of 4 weeks: Group 1 (control group) orally received saline daily for 4 weeks. Group 2 (Cd-treated group) received an oral dose of 5 mg/kg body weight/day CdCl_2 dissolved in distilled water [12]. Group 3

(NSO and Cd treated group) received an oral dose of 1 ml/kg body weight of NSO + 5 mg/kg body weight CdCl_2 [13]. Group 4 (VOO and Cd treated group) were orally given 5 mg/kg body weight/day CdCl_2 + olive oil at a dose of 0.5 ml/kg body weight/day [14].

Sampling and Tissue Preparation

Blood sampling

After administration of the tested compounds, blood samples were collected at the end of the 4 weeks. Blood samples were collected from the retro-orbital plexus of rat's eye in all groups at a fasting state. Blood samples were collected and left to coagulate at room temperature and then centrifuged at $1000 \times g$ for 15 min. The clear non hemolysed supernatant sera were removed and stored at -20°C till used for estimation of biochemical parameters.

Brain and kidney samples

Brain and kidney were quickly excised after dissection of animals. The tissues were weighed and divided into two pieces. The first one was kept in 10% formalin for histopathological investigation, while the second one (0.5 g) was homogenized in ten volumes of (ice-cold phosphate buffered saline PH: 7) until a uniform suspension was obtained. The homogenate was kept in deep freezer at -20°C for catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) assays as well as DNA fragmentation %.

Biochemical Assays

Kits for serum urea [15], creatinine [16] and serum cholinesterase activity [17] were assayed spectrophotometrically. Oxidant/antioxidant status in tissues includes GSH concentration was determined according to the method of Beutler *et al.* [18]. Lipid peroxides as, MDA concentration were measured according to the method of Albro *et al.* [19]. SOD and CAT were measured according to the method described in [20] and [21], respectively. DNA fragmentation % was measured in tissues of brain and kidney according to the method of Burton [22].

Preparation of Histological Sections

Specimens of the brain cortex and kidney were taken and fixed in 10% neutral buffered formalin for 24 h and processed for light microscope. Tissue specimens were embedded in paraffin wax using a conventional method and stained by Harris hematoxylin and eosin stain for histopathological studies [23].

Statistical Analysis

Statistical analysis was carried out using Graph Pad In stat software (Version 3, ISS, Rome, Italy). One-way analysis of variance (ANOVA) test, followed by Tukey-Kramer multiple comparisons post-test were used. The values are expressed

as mean ± standard error. $P < 0.05$ were considered to be statistically significant ($P < 0.05$).

RESULTS

Biochemical Assays

The obtained results in Table 1 showed a marked significant increase ($P < 0.05$) in serum urea and creatinine concentrations and a significant decrease in acetylcholinesterase activity in the Cd treated group compared with control group. NSO and VOO nearly returned these values toward the normal. Comparing Cd treated group with control group revealed a marked significant decrease ($P < 0.05$) in GSH concentration and a significant increase ($P < 0.05$) in MDA concentration, CAT and SOD activities in tissues homogenates obtained from brain and kidney in the Cd treated group compared to control group. NSO and VOO nearly returned these values toward the normal [Tables 2 and 3]. The results in Table 3 showed a marked significant ($P < 0.05$) increase in DNA fragmentation % in brain and kidney in the Cd-treated group compared to control

group. On the other hand, there was a significant ($P < 0.05$) decrease in DNA fragmentation % in brain and kidney in NSO + Cd treated group and VOO + Cd treated group compared with those of the (Cd-treated group).

Histopathological Changes in Brain and Kidney

In the present study, administration of cadmium induced morphopathological changes in the brain involved severe congestion in the blood vessels of the cerebral cortex. The neurons undergo either degenerative or apoptotic changes. The degenerative changes manifested by shrinkage and deeply stained neurons in both studied regions [Figure 1]. It also induced severe damage of renal glomeruli with severe congestion of the renal blood vessels. Marked dilatation of Bauman's capsule and damage of the glomerular epithelium was observed [Figure 2].

NSO and VOO supplementation prevents cadmium-induced degenerative changes in brain and kidney tissues [Figures 1 and 2].

Table 1: Changes in serum urea (mg/dl), creatinine and acetyl cholinesterase activity in different rat groups

Parameters	Urea (mg/dl)	Creatinine (mg/dl)	Acetyl cholinesterase activity (U/L)
Control group	34.5±1.5 ^a	0.9±0.07 ^a	22.19±0.9 ^a
Cd-treated group	68.9±2.8 ^b	2.7±0.1 ^b	5.87±0.6 ^b
NSO+ Cd-treated group	52.6±1.2 ^c	1.8±0.14 ^c	10.38±0.6 ^c
VOO+ Cd-treated group	38.8±1.8 ^a	1.3±0.1 ^a	19.2±1.2 ^a

^{a, b, c}Means in the same row with different superscripts are significantly different ($P < 0.05$). NSO: *Nigella sativa* oil, VOO: virgin olive oil

Table 2: Changes in brain and kidney GSH and MDA concentrations in different rat groups

Parameters	Tissue	GSH (mmol/g tissue)		MDA (nmol/g tissue)	
		Brain	Kidney	Brain	Kidney
Control group		67.14±2.3 ^a	44.86±1.6 ^a	26.8±1.6 ^a	34.37±1.6 ^a
Cd-treated group		23.99±1.6 ^b	9.07±0.7 ^b	55.9±1.4 ^b	89.37±2.4 ^b
NSO+ Cd-treated group		34±1.4 ^c	17.63±1.37 ^c	44.3±1.6 ^c	72.69±2.1 ^c
VOO+ Cd-treated group		66.17±2.1 ^a	41.6±1.09 ^a	27.6±1.3 ^a	38.1±0.74 ^a

^{a, b, c}Means in the same row with different superscripts are significantly different ($P < 0.05$). NSO: *Nigella sativa* oil, VOO: Virgin olive oil, GSH: Reduced glutathione, MDA: Malondialdehyde

Table 3: Changes in brain and kidney CAT, SOD activities and DNA fragmentation % in different rat groups

Parameters	Tissue	CAT (U/g tissue)		SOD (U/g tissue)		DNA fragmentation %	
		Brain	Kidney	Brain	Kidney	Brain	Kidney
Control group		1.8±0.31 ^a	2.46±0.17 ^a	74.24±1.9 ^a	58.49±2 ^a	11.7±0.5 ^a	26.1±1.4 ^a
Cd-treated group		7.8±0.49 ^b	7.37±0.34 ^b	125.3±2.6 ^b	141.9±10.2 ^b	28.2±2.1 ^b	72.17±1.01 ^b
NSO+ Cd-treated group		5.1±0.26 ^c	5.6±0.4 ^c	102.5±3.3 ^c	106.7±1.6 ^c	22.8±1.2 ^c	62.19±1.2 ^c
VOO+ Cd-treated group		1.9±0.29 ^a	3.27±0.25 ^a	77.2±1.4 ^a	62.18±1.4 ^a	15.35±0.9 ^a	26.84±1.6 ^a

^{a, b, c}Means in the same row with different superscripts are significantly different ($P < 0.05$). NSO: *Nigella sativa* oil, VOO: Virgin olive oil, CAT: Catalase, SOD: Superoxide dismutase

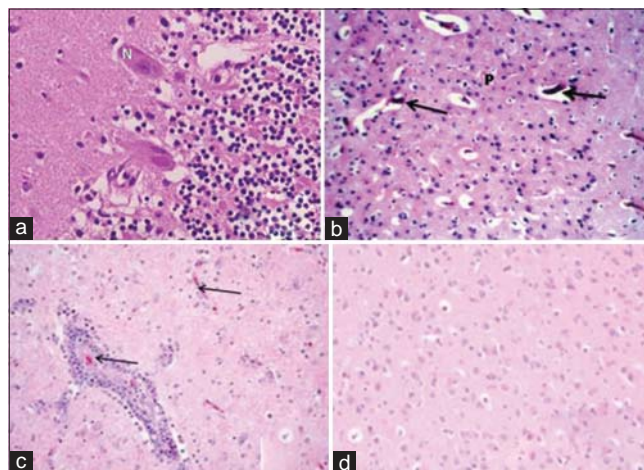


Figure 1: Light micrograph of a cross section in rat brain (cerebral cortex); (1a) control group: Showing normal neurons (N) with no inflammatory cells infiltration (H and E, ×100), (1b) Cd-treated group: Showing severe shrunken pyknotic and darkly stained neurons with multiple vacuolations (P). Congestion in the blood vessels and capillaries (arrow) of cerebral cortex. Degenerative changes of the neurons manifested by shrinkage and deeply stained neurons (S) (H and E, ×100), (1c) *Nigella sativa* + Cd treated group: It showed a little congestion of blood vessels (arrow), local cellular infiltration as compared to that of the Cd treated group (H and E, ×100), (1d) virgin olive oil + Cd treated group: Looks like normal (H and E, ×100)

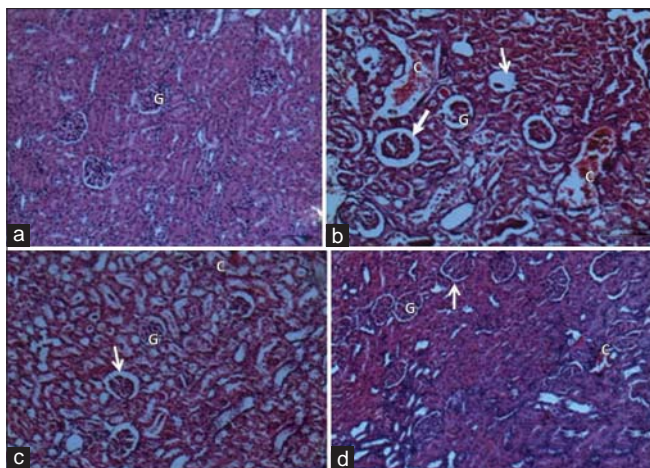


Figure 2: Light micrograph of a cross section in a kidney tissue; (2a) control group: Showing normal glomeruli (G) with no inflammatory reaction and no congestion of blood vessels (H and E, $\times 100$), (2b) Cd-treated group: It showed a severe damage of glomeruli (arrow) and severe congestion of the renal blood vessels (C). Marked dilatation of Bauman's capsule and damage of glomerular epithelium was obvious (arrow head) (H and E, $\times 100$), (2c) *Nigella sativa* oil + Cd-treated group: It showed little congestion (C) and little cellular infiltration when compared to that of Cd treated group (H and E, $\times 100$), (2d) virgin olive oil + Cd-treated group: It showed a little congestion of renal blood vessels (C). There was nearly normal Bauman's capsule (arrow). Little inflammatory cells infiltration was noticed (H and E $\times 100$)

DISCUSSION

Cadmium, a redox-inactive nonessential metal, known to be most toxic environmental pollutant is present in the soil, air, water, cigarette smoke and even in food. It promotes oxidative stress and contributes to the development of serious pathological consequences because of its long retention in some tissues [24]. The mechanism of the cadmium-induced damage includes the production of free radicals that alter mitochondrial activity and genetic information [25]. Therefore, some authors have postulated that antioxidants should be one of the important components of an effective treatment of cadmium poisoning [12]. The present study concentrates on the possible protective effect of NSO, VOO on oxidative damage generated by CdCl_2 induced neurotoxicity and nephrotoxicity.

In this work, the serum level of creatinine and urea was significantly increased after cadmium treatment compared with the control group that was clearly shown in Table 1, indicating the impairment in the kidney functions. Similar observation was obtained by Novelli *et al.* [26]. In fact, urea is the first acute renal marker which increases when the kidney suffers from any kind of injuries. Otherwise, creatinine is the most trustable renal marker and increase only when the majority of renal function is lost [27]. The changes in urea and creatinine level in the present study concluded the severe injured effect of CdCl_2 on kidney.

The decrease in serum acetylcholinesterase activity as shown in Table 1, is in agreement with the observation of El-Demerdash and Elagamy [28] in fish and El-Demerdash *et al.* [29] *in vitro*.

Inactivation of acetyl cholinesterase enzyme, as a result, of the occupation of its active sites by heavy metals has also been suggested by Shaw and Panigrahi [30], however the mechanism of metals underlying the inhibition of acetylcholinesterase is not clear.

The impairment of antioxidant defense system is considered as a critical event in cadmium-induced nephrotoxicity and neurotoxicity. Exposure of cadmium is characterized by the depletion of tissue and circulating non-enzymatic antioxidants including GSH [31]. In the current study, lipid peroxidation level was estimated by the measurement of MDA, its level was significantly elevated in brain and kidney tissues of rats treated with cadmium compared to the control group [Table 2] thus suggesting increased oxidative stress. These results were supported by Manca *et al.* [32] who reported that lipid peroxidation is an early and sensitive consequence of cadmium exposure. In addition, Kawamoto *et al.* [33] reported that cadmium is thought to induce lipid peroxidation and this has often been considered to be the main cause of its deleterious influence on membrane-dependent function.

In the agreement with a previous study of Koyuturk *et al.* [34], the level of GSH was significantly decreased in the brain and kidney tissues of cadmium treated group compared to the control group [Table 2]. This decrease in GSH contents may be due to its consumption in the prevention of free radical-mediated lipid peroxidation [34]. Also, GSH may be consumed in the detoxification of heavy metals [35]. Furthermore, it has suggested that the decrease in GSH concentrations upon cadmium exposure might impair the degradation of lipid peroxides, thereby leading to its accumulation in the target organs [36]. In the controversy to the current results, Kamiyama *et al.* [37] reported an increase in GSH concentrations in liver and kidney tissues after the cadmium injection which could be explained as a protective mechanism. Furthermore, Rana and Verma, [38] had reported that, GSH is an important antioxidant defense, which forms complexes with cadmium through the free sulfhydryl group and thereby alters cadmium distribution and excretion.

In the present investigation, the activities of SOD and CAT were increased significantly in rats administrated with cadmium [Table 3], which is in line with the report of [39]. SOD and CAT constitute a supportive team of defense against reactive oxygen species. Our results partially agree with Erejuwa *et al.* [40] who reported that SOD activity was significantly elevated, while CAT activity was significantly reduced in rat renal homogenate. In the controversy to the current results, Renugadevi and Prabu [41] reported a decrease in CAT and SOD activities in rats exposed to oxidative stress. SOD converts superoxide anion (O_2^-) to hydrogen peroxide H_2O_2 [42] which are subsequently converted to water and molecular oxygen by glutathione peroxidase or CAT [43]. Thus, it could be suggested that the present increase in SOD activity after 4 weeks of CdCl_2 ingestion may be considered as a defense mechanism of the cortical neurons and renal cells against the increase in the production of superoxide anions during this current state of oxidative stress. CAT catalyzes the conversion of H_2O_2 to H_2O

and molecular oxygen [43]. Hence, an increase in CAT activity after 4 weeks of CdCl₂ treatment could be expected in order to convert H₂O₂, which produced, as a result, of the enzymatic activity of SOD, to water and molecular oxygen. Our result agreed with recent studies conducted by Mourad and Noor [44] where they reported that there was an increase in CAT and SOD activities in rats administrated aspartame. Moreover, it is reported that CdCl₂ often generates free radicals, which in turn activate O₂ and produce reactive oxygen species, including hydroxyl radicals, singlet oxygen, superoxide and hydrogen peroxide and consequently lead to DNA damage.

A significant increase in the DNA % damage was observed in the brain and kidney tissues of rats treated with cadmium compared with the control group [Table 3]. MDA, 4-hydroxy-2-nonenol and several reactive mutagenic and genotoxic lipid peroxidation products bind to DNA causing strand breakage and distortion [45], which is in line with the present findings. This might be a major reason for increased severity of DNA damage in cadmium treated group.

In the present study, administration of cadmium 5 mg/kg orally daily for 4 weeks induced significant damage in function and structure of the kidney assessed by increased serum creatinine and urea concentrations and existence of severe damage of glomeruli with severe congestion of the renal blood vessels. These results agreed with Damek-Poprawa and Sawicka-Kapusta [46] who found an atrophy of some glomerular capillaries as well as proximal tubular necrosis and apoptosis, degenerative changes with pyknotic nuclei, and cytoplasmic vacuolation in the distal convoluted tubules. Epidemiological studies have revealed that cadmium is one of the most toxic of the heavy metals to humans; 70% of the ultrafiltered cadmium is taken up, largely by the proximal tubules of the kidney and is accumulated mainly in kidney cortex leading to proximal tubular lesions [47]. These findings are in agreement with the current study. The nephrotoxicity of cadmium has been extensively studied in various experimental models [48].

Administration of cadmium-induced morphopathological changes in the brain involved different types of neurons in the examined region (cerebral cortex) with sever congestion in the blood vessels of the cerebral cortex. The neurons undergo either degenerative or apoptotic changes. The degenerative changes manifested by shrinkage and deeply stained neurons in both studied region. These observations are in harmony with experimental studies in the central nervous system of newborn rats and rabbits exposed to high doses of cadmium, which showed extensive hemorrhage in the cerebral and cerebellar cortices, several pyramidal cells with pyknotic nuclei, neuroglial cells with the cytolysis and altered purkinje cells [49]. It was reported that cadmium has distinct neurotoxic effects in adults and newborn animals. In adult rats, high doses of cadmium produce damage to the Gasserian and sensory spinal ganglia, but in newborn rats, high doses of cadmium produce severe hemorrhagic lesions in cerebrum and cerebellum [50]. Furthermore, cadmium is a highly toxic metal; in particular, it was reported to produce neuropathological and neurochemical alterations in the central nervous system resulting in irritability

and hyperactivity [6]. The necrotic and degenerative changes of brain may be attributed to the high susceptibility of rats to cadmium toxicity, and it was considered an important indication for the neuro-toxic effect of cadmium due to oxidative stress induction. This interpretation is confirmed by Williams [51] who reported that oxidative stress is one of the mechanisms that contribute to structural changes, and it plays an important role in neurodegeneration.

Treatment with NSO significantly declined the effects of cadmium-induced damage in the kidney tissues, and it was evidenced by the decreased level of creatinine and blood urea [Table 1]. This is in agreement with Al-Okbi *et al.* [52].

Our result also showed that the administration of NSO effectively led to an increase in the GSH level, decrease in the MDA level [Table 2] and improve or decrease of DNA fragmentation in the kidney tissue [Table 3] and this agreed with Sayed-Ahmed and Nagi [53] who have investigated the possible protective effects of thymoquinone (TQ), a compound derived from NS with potent anti-oxidant activity, against gentamycin-induced nephrotoxicity. Supplementation with TQ resulted in a significant increase in GSH and decreased in blood urea nitrogen and creatinine, these results coincide with our results. The current study showed that administration of NSO with CdCl₂ exerts an important protective effect as it corrected the oxidative stresses and returned CAT and SOD to nearly normal level in kidney tissues in compared with cadmium treated group. The experiments discussed here showed that NSO were able to rescue cells from oxidative stress-induced cell damage. There are three alternative explanations for the protective effect of NSO against oxidative stress [54]. They may act as direct antioxidants, block reactive oxygen species generation by inhibiting a step in the programmed cell death pathway (apoptosis), or directly cause a low level of reactive oxygen species production that rapidly induces a reactive oxygen species defense system before the glutamate-induced cell death program is complete. The last is a type of preconditioning that could be caused by the exposure of cells to reactive oxygen species lowering material [55].

Histopathological examination of kidney tissues confirmed the biochemical data where NSO supplementation prevents cadmium-induced degenerative changes in kidney tissues, suggesting that these effects may be related to the ability of TQ to modulate cellular oxidative stress.

Treatment with NSO in the present study significantly restored the brain tissue level of enzymatic antioxidants (CAT and SOD), GSH and MDA compared to cadmium treated rats. This might indicate the usefulness of NSO, as an excellent source of antioxidants, in modulating cadmium-induced neurotoxicity. The protective effect of antioxidant-rich diets in diseases involving oxidative damage has been reported. Burits and Bucar [56] showed that NS have appreciable antioxidant and free radical scavenger properties. Yaman and Balıkcı [57] showed that NSO and TQ may have protective effects on lipid peroxidation process during ischemia–reperfusion injury in rat hippocampus [58]. Al-Majed *et al.* [59] have evaluated

the neuroprotective effect of NSO against transient forebrain ischemia-induced neuronal damage in the rat hippocampus. Treatment of ischemic rats with the NSO decreased the elevated levels of MDA, CAT and SOD activities to normal levels, and it increased the level GSH. TQ and its reduced product, toluhydroquinone, inhibited the *in vitro* non-enzymatic lipid peroxidation in hippocampal homogenate induced by iron-ascorbate. Also Mansour *et al.* [60] reported that, administration of NSO or TQ, elicited marked antioxidant effect by a profound decrease in the antioxidant enzyme activities (CAT and SOD). The histopathological details appeared more normal comparing with cadmium treated group. That protection makes NSO a promising agent in pathologies implicating neurodegeneration. TQ, the active constituent of NS, has been well documented as a potent antioxidant, particularly against the CCl₄-induced free radical species [61] and lead acetate-induced hepatic damage in mice [62]. TQ prevents the formation of stable toxic complex by a combination of CCl₄ and reactive oxygen species and the glycolipid component of the cell membrane, and, therefore, restores cellular architecture [63].

The present biochemical and histological results proved that NS seed oil possess potential to protect the brain and kidney tissue against oxidative damages and could be used as an effective protector against cadmium-induced brain and renal damages.

Our study indicates the beneficial effects of VOO in combating nephrotoxicity, as the levels of urea and creatinine decreased from higher levels in cadmium treated group to approximately normal levels in co-treatment of olive oil and cadmium. These data are in agreement with previous reports, which proved that the beneficial effects of VOO were evidenced by reduced plasma urea and creatinine concentrations in the group receiving olive oil compared to the non-oil treated animals receiving gentamycin only [64].

The oral administration of VOO with CdCl₂ for 4 weeks caused some significant improvement in CdCl₂-induced antioxidant defense by normalizing the antioxidant activity of the enzymes (CAT and SOD), GSH and reducing MDA levels in brain and renal tissues. Moreover, the oral supplementation of olive oil to rats restored the damage caused to the liver by inhibiting lipid peroxidation and improving enzymatic activities [65]. The mechanism proposed to explain the beneficial effects of olive oil may be attributed to its highest content of MUFA, mainly oleic acid which has different effects on lipid profiles and peroxidation in rabbit liver mitochondria [66]. VOO contains a considerable amount of oleuropein, tyrosol, hydroxytyrosol and caffeic acids which all have potent inhibiting effects against reactive oxygen species [67]. Hydroxytyrosol is highly effective against DNA damage induced by peroxynitrite *in vitro* [68]. Caffeic acid phenethyl ester and its related compounds decrease the functional alterations of the isolated mouse liver mitochondria submitted to *in vitro* anoxia-reoxygenation [67]. The co-administration of VOO and CdCl₂ in rats restored the altered levels of serum acetylcholinesterase as well as brain and renal DNA fragmentation % to approximately their levels in the control group; it also significantly improved the tissue level of enzymatic antioxidants compared to cadmium treated rats.

These effects have been attributed to its high MUFA content, mainly the oleic acid and polyunsaturated fatty acids content. The results of histopathological examination of rat brain and renal tissues confirmed our biochemical findings.

Unfortunately, we did not find data concerning the effect of VOO on heavy metals induced neurotoxicity to discuss them with our results. These results concerning the ability of VOO to ameliorate the deleterious effects of CdCl₂ on brain tissues are promising and novel.

CONCLUSION

From our results, it could be concluded that, administration of NSO or VOO with CdCl₂ succeeded in amelioration and improvement of the altered biochemical and oxidative-antioxidant parameters as well as DNA fragmentation % to nearly those of the control group. Our histopathological studies of brain and kidney tissues confirmed the alterations caused by CdCl₂ toxicity and the success of NSO and VOO treatment in ameliorations of organ damage. Collectively, these results indicate that VOO and NSO can be used as potentially effective therapeutic agents for workers in factories that their products are contaminated with Cd particularly cement factories.

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