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Protective effect of Nigella sativa oil on cadmium-induced hepato-nephrotoxicity

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Abstract

Cadmium (Cd) is a toxic xenobiotic from the heavy metals family. It is primarily accumulated in the liver and kidneys, where it has adverse effects. This study aimed to investigate the protective effect of *Nigella sativa* oil (NSO) consumption against Cd hepato-nephrotoxicity in rats. Male albino Wistar rats were separated into four groups of 5 animals each and dealt orally with NSO (4%) alone or accompanied by cadmium (50 mg/l) for two months. The results revealed that Cd exposure caused weight loss and increased serum transaminases, uric acid, creatinine, urea, and kidney weight. It significantly increased malondialdehyde (MDA) and carbonyl proteins in both liver and kidneys, while a marked reduction in tissue glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) was observed. Combined treatment with NSO improved body weight, liver and kidney function, reduced oxidative stress, and enhanced the antioxidant potential by reducing MDA and carbonyl proteins. These findings suggest that NSO consumption may protect rats' liver and kidney function against injury caused by Cd; this is achieved by increasing antioxidant enzyme activities and reducing oxidative stress.

Keywords: Cadmium; hepato-nephrotoxicity; *Nigella sativa*; oxidative stress.

1. Introduction

Heavy metal accumulation results from the industry's growth and a sharp increase in industrial waste released into the environment, mainly in soil and water **(Dixit et al., 2015).** Some metals, such as cadmium (Cd), nickel, mercury, and lead, are toxic even in trace amounts **(Rahman and Singh, 2019).** Exposure to these toxic heavy metals causes health problems for animals and humans **(Schaefer et al., 2023).** An increased risk of atherosclerosis, hypertension, type 2 diabetes, and some cancers has been linked to Cd **(Urbano et al., 2023).** Oxidative stress is another effect of Cd that results in an imbalance between antioxidants and free radicals and can lead to disruption of cellular macromolecules such as lipids, proteins, and DNA and the peroxidation of membrane fatty acids **(Chaudhary et al., 2015).**

The long-term bioaccumulation of Cd (20 to 40 years) significantly damages the various organs. When first exposed, the liver primarily absorbs and stores most of the Cd. It causes mitochondrial malfunction, oxidative stress, and a change in the mitochondria's permeability. Cd hepatotoxicity is followed by secondary damage. It causes hepatocellular damage by degrading endothelial cells **(Ghoneim et al., 2015).**

At the kidney's level, Cd produces reactive oxygen species (ROS), which cause inflammation, apoptosis, and other changes in kidney tissues, which results in nephrotoxicity. Kidney failure results from exposure to Cd, which affects glomerulus filtration rates. After long-term exposure, cadmium attains the renal cortex and primarily damages the proximal tubular epithelium **(Mohammed et al., 2014).**

In recent years, researchers have tended to find pharmacological solutions to cadmium accumulation and damage. Chelating agents have been the subject of several studies and can be administered intravenously, intramuscularly, or orally **(Bamonti et al., 2011).** However, chelation therapy can produce toxic effects because all of these agents have minor and potentially fatal side effects, including damage to the kidneys, an abnormal heartbeat, and swollen veins **(Flora and Pachauri, 2010).**

Given these disappointing results, many researchers have turned to traditional medicine and herbal medicine in search of functional food ingredients and/or plant extracts that are easily accessible and inexpensive. Among the most used medicinal plants, we find *Nigella sativa* L. has been paramount among the most used medicinal plants for more than 3000 years **(Burdock, 2022).** An annual flowering plant in the Ranunculaceae family, *Nigella sativa*, is one such food source that has a wide range of biological characteristics, such as hepatoprotective, nephroprotective, anti-inflammatory, and antioxidant effects **(Alam et al., 2023).**

Many samples of NS seeds and their extracts have been analyzed and characterized regarding their physical properties, chemical compounds, minerals, and lipid compounds (unsaturated fatty acids, phytosterols, and quinones). These extracts contain vitamins, flavonoids, and other polyphenols **(Akram Khan and Afzal, 2016)**. Its phenolic compounds are the subject of much research. They are widely used in therapeutics as enzyme inhibitors, antioxidants, and antiradicals (Sivapriya and Leela, 2007). In addition, its active ingredient is thymoquinone (TQ), which constitutes 30 to 48% of NSO **(Daryabeygi-Khotbehsara et al., 2017).** It has the antioxidant capacity to inhibit, in the liposome, lipid peroxidation without enzymes **(Burits and Bucar, 2000).** Furthermore, no toxicological or significant side effects were reported in human **(Heshmati and Namazi, 2015)** and animal **(Zaoui et al., 2002)** studies. Our work aims to study the protective effect of fixed oil extracted from *Nigella sativa* seeds against Cd's toxic and harmful effects on the liver and kidneys of Wistar rats.

2. Materials and Methods

2.1. Chemicals

Cadmium chloride ($CdCl_2$, 98% purity) was obtained from Sigma-Aldrich Company (United States). This research also used other analytical-grade substances.

2.2. Vegetable oils

The following procedure was used to extract the seed oil of NS: Using a crusher (Retsch RM 100), seeds from Tlemcen, Algeria, were ground. Petroleum ether (40–60 °C) was used in a Soxhlet system for two hours to extract the lipid fraction (Natural Products Laboratory, Tlemcen, Algeria).

After the solvent's evaporation, the lipid fraction's residues were weighed. The percentage of oil in the nigella seeds is 34%. Sunflower oil was purchased locally (Fleurial Plus, Algeria).

2.3. Experimental animals

The breeding of the animals took place in the animal house of the biology department. The Tlemcen University Ethics Committee for Animal Experimentation approved the use of animals in this research.

Male Wistar rats (Iffa Credo, France), aged one month, were kept individually in stainless-steel cages with a 12-hour light/dark cycle, a controlled temperature of 23 ± 1 °C, and a relative humidity of 50 ± 10%. They were also provided with unlimited access to food and water. Four groups of five rats each were created out of the animals.

The control rats (group 1) were given sunflower oil (diet 1) and normal water. Rats in group 2 received a diet that included nigella oil (diet 2) and was maintained with normal water. Rats in group 3 were fed diet 1, and their drinking water was contaminated with $CdCl_2$ (50 mg/l). Rats in group 4 were fed diet 2 and drank $CdCl_2$-contaminated water (50 mg/l). Rats' body weight increase was tracked throughout the two-month trial. Table 1 indicates the typical oil composition of the experimental diets.

Table 1: Diet composition used in experiments.

Constituents (g/100 g)	**Diet 1**	**Diet 2**
Casein	16	16
Methionine	0.3	0.3
Starch	55.7	55.7
Cellulose	5	5
Saccharose	5	5
Vitamin mix	2	2
Mineral mix	7.37	7.37
Oil	4	4
Total	100	100
Oil composition (%)		
Myristate	0	0.2
Palmitate	17.2	11.7
Stearate	5	3.2
Oleate	60.02	24
Linolenate	1.9	0.3
Linoleate	11.98	56.4

Arachidonate	1.9	0.2
Tocopherol (mg/100 g)	76.34	280.1

Composition of the mineral mix (g/100 g of dry diet): Mg^{2+}: 0.4; Fe^{2+}: 0.12; K^{+}: 2.4; Na^{+}: 1.6; Ca^{2+}: 4. Composition of the vitamin mix (mg/kg of dry diet): Ascorbic acid: 240, α-tocopherol: 51, nicotinic acid: 30, folic acid: 1.5, biotin: 0.09, pantothenic acid: 21, inositol: 5, retinol: 1.8, cholicalciferol: 0.019, thiamine: 6, riboflavin: 4.5. Trace elements: manganese: 0.032; copper: 0.05; zinc: 0.018.

2.4. Sampling and tissue preparation

After two months of diet, the control and treated groups were sacrificed by anesthesia after being injected with chloral hydrate (10%) (3 mL/kg body weight) intraperitoneally. Blood samples were gathered in tubes containing heparin. After centrifugation (1600 × g) for 10 minutes, the plasma was separated and stored in minuscule aliquots at -20 °C until analysis.

Immediately removed, the liver and kidneys were rinsed in ice-cold saline buffer (20 mM Tris-HCl, 0.14 M NaCl buffer, pH 7.4), weighed, and then diced finely in the same solution before being homogenized (10% w/v) in a Potter Elvehjem homogenizer. Homogenate aliquots were saved for later biochemical analysis, and the homogenate tissue was utilized for an instantaneous lipid peroxidation test.

2.5. Mitochondrial fraction extraction

The procedure for isolating hepatic and renal mitochondria is based on a differential centrifugation cell fractioning technique described by **Frezza et al. (2007)**. 2 g of each organ are cut into small pieces and homogenized in a cold solution of Tris buffer (10 mM, pH 7.4) containing sucrose using a teflon glass potter. A first centrifugation (BEEKMAN, ROTOR JA17) for 10 minutes at 600 x g at 4 °C allows for the removal of cellular debris. The supernatant is recentrifugated at 7000 x g for 15 minutes at 4 °C to recover the mitochondria in the form of a brown ass. The rosy layer of microsomes floating on the buttock is removed in order to obtain purified mitochondria. The pillow is then suspended again in 2 ml of tampon solution specific to the dosing parameter.

2.6. Measurement of total plasma fatty acids

Gas chromatography (CG) was used to assess the fatty acid composition of plasma. After modifying the Bligh and Dyer process, fatty acids were trans-esterified into methyl esters (FAMEs) using 14% boron trifluoride in methanol. Using a capillary column (Alltech EC-Wax) with a flame ionization detector (FID) and a film thickness of 30 m×0.53 mm×1.2 µm, FAMEs were detected by CG Varian CP-3380.

The carrier gas employed was helium. One microliter (1 µL) was injected, and the oven temperature was maintained at 250 °C. After two minutes at 180°C, the temperature was raised to 220°C at 6°C/min. Following this time, the temperature was maintained for ten minutes at 220°C. FAMEs were detected in triplicate under identical conditions as the plasma FAMEs using the authentic FAMEs standard (Grace AOCS Mix 3A).

2.7. Liver and kidney function investigation

Enzymatic kits from Sigma were used to measure biochemical indicators in plasma, such as urea, creatinine, aspartate aminotransferase, alanine aminotransferase, and uric acid.

2.8. Measurement of renal and hepatic oxidative markers

GSH concentration and other antioxidant and oxidant status in tissues were assessed using the **(Curello et al., 1987)** approach. The Albro et al. **(Albro et al., 1986)** technique determined lipid peroxides and MDA concentrations. Carbonyl groups were quantified based on their reactivity with dinitrophenylhydrazine (DNPH) to determine the extent of oxidative damage to proteins **(Levine et al., 1990).** Methods outlined by **Nishikimi et al. 1972** and **Aebi ., 1984** for measuring SOD and CAT were used.

2.9. Statistical analysis

The mean ± standard deviation was used to express the results. After utilizing a one-way ANOVA, Tukey's test was employed to assess all the results. $P < 0.05$ values were regarded as statistically significant. The values denoted by distinct superscript letters (a, b, and c) exhibit a highly significant difference ($P < 0.01$). According to the results, this value is predominant.

3. Results

3.1. Body weight and average Cd intake

In comparison to the control group (group 1), oral administration of Cd or NSO alone resulted in a considerable decrease in body weight of approximately 25.55% and 18.10%, respectively, as shown in Fig. 1. However, rats who received Cd with NSO supplementation (group 4) did not have a decrease in body weight compared to rats that received the same regiment without Cd. Estimating water consumption, the average Cd intake for groups 3 and 4 was 330 ± 50 µg/24 h/rat and 380 ± 72 µg/24 h/rat, respectively (Fig. 2).

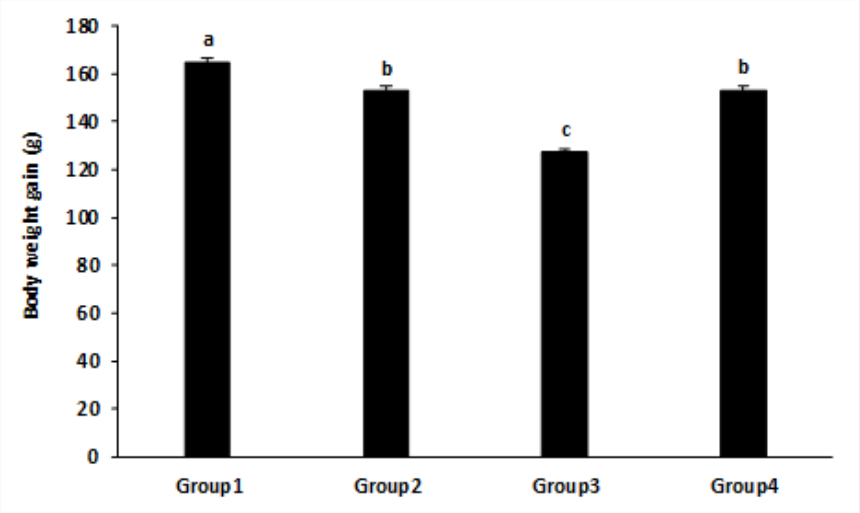

Fig. 1. Effects of NSO and/or cadmium alone on body weight gain following eight weeks of experimentation. The results are expressed as the mean ± SD. Values with different superscript letters (a, b, c) are very significantly different (P < 0.01).

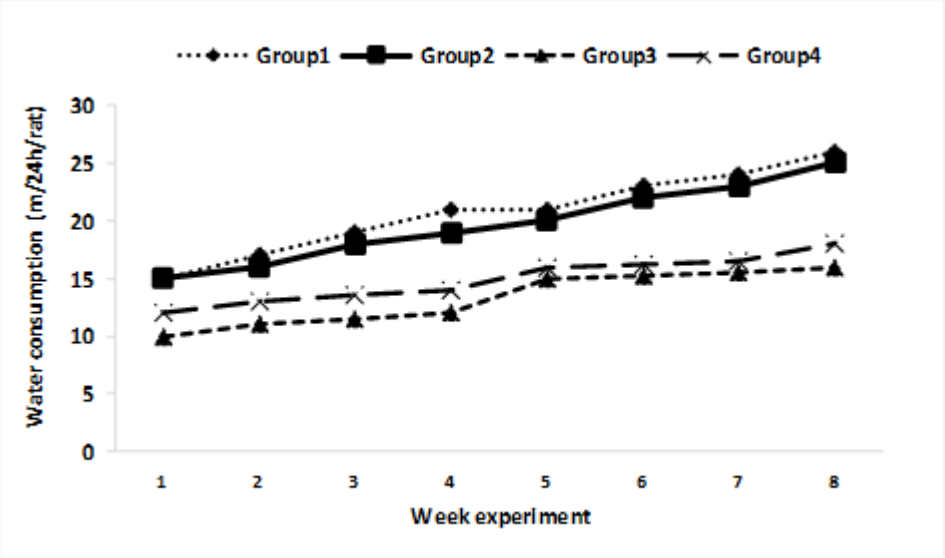

Fig. 2. Drinking water consumption in rats exposed to Cd. The results are expressed as the mean ± SD. Values with different superscript letters (a, b, c) are very significantly different (P < 0.01).

3.2. Plasma fatty acid composition

Table 2 displays the plasma fatty acid composition. The treated group's levels of linoleic acid (C18:2) and eicosenoic acid (C20:1) were reduced compared to the control. There was no discernible change in other fatty acids. On the other hand, NSO treatment increased the percentage of linoleic acid (23.34%) in rats given Cd.

Table 2: Effects of cadmium and NSO on the composition of plasma fatty acids (wt%).

Fatty acid	Group 1	Group 2	Group 3	Group 4
C16:0	25.81 ± 1.43^{a}	19.87 ± 1.32^{b}	24.23 ± 2.54^{a}	26.76 ± 1.32^{a}
C18:0	19.32 ± 2.53^{a}	15.21 ± 1.65^{b}	21.32 ± 1.76^{a}	16.43 ± 1.43^{a}
C18:1	30.65 ± 1.09^{a}	28.65 ± 1.56^{a}	29.76 ± 1.23^{a}	29.70 ± 1.76^{a}
C18:2	12.11 ± 0.7^{c}	29.54 ± 1.06^{a}	9.04 ± 0.65^{d}	23.34 ± 0.32^{b}
C18:3	0.77 ± 0.05^{a}	0.34 ± 0.05^{b}	0.76 ± 0.1^{a}	0.32 ± 0.04^{b}
C20:1	0.47 ± 0.06^{a}	ND	0.12 ± 0.07^{b}	ND
C22:0	ND	0.76 ± 0.11^{a}	ND	0.80 ± 0.12^{a}
C20:4	6.43 ± 0.06^{a}	ND	7.65 ± 0.54^{a}	ND
∑SFA	45.13 ± 3.98^{a}	35.08 ± 3.08^{b}	45.55 ± 4.3^{a}	43.99 ± 2.87^{a}
∑MUFA	31.12 ± 1.15^{a}	28.65 ± 1.56^{a}	29.88 ± 1.3^{a}	29.70 ± 1.76^{a}
∑PUFA	19.31 ± 0.81^{c}	29.88 ± 1.11^{a}	17.45 ± 1.29^{c}	23.66 ± 0.36^{b}

Fatty acids: number of carbon atoms: number of double bonds, followed by the position of the first double bond relative to the fatty acid's methyl end (n-). ∑SFA: Sum of the saturated fatty acids; ∑MUFA: Sum of the mono-unsaturated fatty acids; ∑PUFA: Sum of the polyunsaturated fatty acids. The results are expressed as the mean ± SD. Values with different superscript letters (a, b, c) are very significantly different ($P < 0.01$).

3.3. Organ weight and biochemical parameters

Compared to the other groups, the addition of Cd significantly increased kidney weight. However, NSO had a corrective effect on kidney weight in Cd-treated rats. No differences were observed in liver weight between the groups of rats under investigation (Table 3). Rats administered oral Cd showed altered liver function. Group 3 had considerably higher ($P < 0.01$) levels of both alanine transaminase (ALT) and aspartate transaminase (AST). NSO treatment prevented the increase in plasma transaminase levels in group 4.

Plasma urea, creatinine, and uric acid levels in cadmium-treated rats fed diet 1 increased significantly ($P < 0.01$). The administration of NSO improves these values.

Table 3: Effects of NSO and cadmium on organ weight and biochemical parameters.

Parameters	Group 1	Group 2	Group 3	Group 4
Liver weight	3.14 ± 0.15^{a}	3.15 ± 0.15^{a}	3.12 ± 0.16^{a}	3.12 ± 0.11^{a}
AST (U/L)	80.32 ± 1.3^{b}	81 ± 1.34^{b}	118.65 ± 1.65^{a}	$8a3.45 \pm 1.54^{b}$
ALT (U/L)	38.76 ± 0.65^{b}	39.43 ± 1.09^{b}	58.54 ± 1.54^{a}	35.54 ± 1.21^{c}
kidney weight	0.37 ± 0.05^{a}	0.37 ± 0.03^{a}	0.54 ± 0.03^{b}	0.36 ± 0.02^{a}
Urea (mg/dL)	36.14 ± 1.50^{b}	35.32 ± 1.32^{b}	64.76 ± 1.21^{a}	38.65 ± 1.83^{b}
Creatinine (mg/dL)	0.73 ± 0.08^{b}	0.69 ± 0.15^{b}	1.87 ± 0.04^{a}	0.82 ± 0.14^{b}
Uric acid (mg/dL)	1.76 ± 0.12^{b}	1.54 ± 0.23^{b}	3.56 ± 0.09^{a}	1.66 ± 0.17^{b}

The results are expressed as the mean ± SD. Values with different superscript letters (a, b, c) are very significantly different ($P < 0.01$)

3.4. Oxidative stress biomarkers

Tissue homogenates from the liver and kidney showed a significant increase in the level of MDA and carbonylated proteins and a significant decrease in the level of GSH, CAT, and SOD activities in group 3 compared to the control group. The introduction of NSO has improved these values (Table 4).

Mitochondrial fractions from the liver and kidneys showed a significant increase ($P < 0.01$) in the levels of MDA, carbonylated proteins, hydroperoxides, and conjugated dienes and a significant decrease ($P< 0.01$) in the level of GSH, CAT, and SOD activity in the Cd group (Group 3) compared to the control group. The introduction of NS oil improved these values. However, our results showed no significant difference in Mn-SOD activity in CD-intoxicated rats (Table 4).

Table 4: Oxidative stress markers in rat groups' livers and kidneys.

Groups	CAT (U/ mg prot)		SOD (U/mg prot)		GSH (µmol/g prot)		MDA (µmol/g prot)		Protein carbonyls (µmol/g prot)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
1	80.65 ± 1.43[a]	60.25 ± 1.23[a]	65.54 ± 2.43[a]	25.14 ± 2.43[a]	24 ± 2.6	0.87 ± 0.	0.45 ± 0.12[b]	0.25 ± 0.	1.60 ± 0.12[b]	0.73 ± 0.
2	79.76 ± 1.54[a]	59.26 ± 1.22[a]	63.43 ± 2.12[a]	23.39 ± 2.12[a]	26 ± 1.3	1.09 ± 0.	0.55 ± 0.13[b]	0.26 ± 0.	1.61 ± 0.11[b]	0.71 ± 0.
3	43.65 ± 2.22[c]	31.60 ± 1.02[c]	41.98 ± 2.87[c]	19.08 ± 2.07[c]	15 ± 3.6	0.55 ± 0.	0.73 ± 0	0.83 ± 0.	3.54 ± 0.20[a]	1.24 ± 0.
4	60.76 ± 1.76[b]	43.25 ± 1.16[b]	59.23 ± 1.12[b]	20.03 ± 1.04[b]	27.06 ±2.54[a]	0.76 ± 0.	0.52 ± 0.05[b]	0.32 ± 0.	1.61 ± 0.13[b]	0.81 ± 0.
	Liver M	Kidney Mito	Liver M	Kidney Mito	Liver M	Kidney Mito	Liver M	Kidney Mito	Liver Mito	Kidney Mito
1			30,85 ± 1,23[a]	17,15 ± 1,11[a]	4,94 ± 0,03[b]	2,10 ± 0,	17,54 ± 1,65[c]	8,87 ± 0.	3,22 ± 0,12[c]	1,05 ± 0,
2			30,96 ± 1,22[a]	16,86 ± 1,23[a]	6,03 ± 0,15[a]	3,19 ± 0,	17,12 ± 1,26[c]	9,01 ± 0.	3,35 ± 0,13[c]	0,99 ± 0,
3			30,65 ± 1,12[a]	17,20 ± 1,05[a]	3,18 ± 0,07[d]	1,02 ± 0,	23,5 ± 2	12,58 0.20[a]	6,44 ± 0,11[a]	3,22 ± 0,
4			30,76 ± 1,06[a]	16,85 1,06[a]	4,03 ± 0,12[c]	1,53 ± 0,	18,64 ±1,04[b]	10,76 0.11[b]	4,66 ± 0,09[b]	2,12 ± 0,

Mito :mitochondria GSH: glutathione peroxidase; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase. The results are expressed as the mean ± SD. Values with different superscript letters (a, b, c) are very significantly different ($P < 0.01$).

4. Discussion

In this study, we chose Cd as a model heavy metal ubiquitous in the environment, as it is the cause of many pathologies, such as liver and kidney damage, and its long-term bioaccumulation in the food chain **(Ezedom et al., 2020).** According to our results, consuming 50 mg/l of oral Cd-polluted drinking water for two months caused an apparent reduction in body weight, indicating toxicity. Several studies agree with our results **(Amamou et al., 2015; Gaurav et al., 2010).** Lipid peroxidation may be a reference for this decrease **(Gaurav et al., 2010).** Indeed, Cd has a cytotoxic effect on intestinal epithelia, causing peroxidation of membrane lipids, which leads to a decrease in intestinal absorption **(Berzina et al., 2007).** A recent study found intestinal epithelial damage through histopathological examination of the duodenum and ileum in rats exposed to Cd. This damage proved that impaired digestion and nutrient absorption may have contributed to body weight loss **(Akinrinde et al., 2022).**

On the other hand, this work confirms the preventive effect of NSO on rats' weight recovery. Tayman et al. **(Tayman et al., 2012)** also showed the beneficial effects of NSO treatment in attenuating intestinal damage, correcting lipid peroxidation, and achieving significant weight gain in rats with necrotizing enterocolitis.

Rats receiving Cd showed a reduction in serum levels of linoleic and gadoleic acid without altering the proportion of eicosenoic acid (20:4). Amamou et al. **(Amamou et al., 2015)** showed that while the relative amount of arachidonic acid remained unchanged, the presence of Cd in the intestines reduced

the absorption of linoleic acid. However, our results showed improved linoleic acid content in rats receiving Cd and NSO supplementation. Linoleic acid is the primary fatty acid in NSO, accounting for 56.4% (Table 1), and the increase in serum linoleic acid could reflect its consumption. The high unsaturated fatty acid content signifies that it offers considerable resistance to oxidative rancidity during storage and health benefits when used. Linoleic acid is a critical polyunsaturated fatty acid used in the biosynthesis of arachidonic acid and some prostaglandins. It is found in cell membranes and has multiple benefits, such as providing antioxidant defense, improving blood cells, stabilizing glucose levels, and reducing body fat **(Farhan et al., 2021).**

Furthermore, the liver is the principal organ for the biotransformation of toxic substances. Our study indicated a significant increase in average concentrations of transaminases (ASAT and ALAT) in intoxicated rats, which is in agreement with those of Brzóska et al. **(Brzóska et al., 2003)** and Nemmiche et al**. (Nemmiche et al., 2007).** The disruption of the hepatic balance, expressed by the increase of transaminases, in rats intoxicated by cadmium indicates a liver injury that can be explained by the leakage of tissue enzymes into the plasma, which is due to membrane permeability alteration **(Kehili et al., 2017).**

However, transaminase levels in rats exposed to Cd were reduced significantly after NSO administration. Previous study have shown the hepatoprotective effect of TQ, which has reduced levels of ALT in hepatocytes isolated and oxidized by tertbutyl hydroperoxide (TBHP) **(Soudani et al., 2011).** An analysis of the impact of different components of nigella extracts (TQ, p-cymene, and α-opinion) on hepatotoxicity in rats also showed that only TQ has a hepatoprotective effect **(El-Dakhakhny et al., 2000).**

Kidneys accumulate a significant amount of cadmium, which can severely damage them with prolonged exposure and cause renal tubular degeneration **(Azzaz et al., 2022; Schaefer et al., 2022).** In our work, urea, creatinine, and uric acid values and the relative weight of the kidneys were significantly increased in rats treated with cadmium alone, indicating impaired renal functions. **Badawy et al. (2023)**, reported the same findings after 4 and 8 weeks of administering oral gavage (5 mg Cd/kg body weight) to intoxicated rats twice a week. The increase in urea and creatinine levels observed in rats may be caused by cadmium binding with metallothionein in the liver. This binding causes cadmium to be released into the plasma, which is then filtered in the glomerular and re-absorbed by the kidney's proximal tubules, ultimately leading to damage in the renal tissues **(Johri et al., 2010).**

Uric acid is primarily synthesized in the liver via xanthine oxidoreductase, then undergoes glomerular filtration, tubular reabsorption, and renal excretion. This elimination process involves basolateral uptake facilitated by an organic anion transporter, followed by efflux mediated by multidrug resistance protein and the urate transporter **(Sun et al., 2017).** Cadmium-induced renal damage begins with proximal renal tubular injury preceding glomerular impairment. Tubular organic anion uptake transporters are potential targets for cadmium toxicity, resulting in reduced basolateral invaginations and down-regulation of organic anion and cation transporters, ultimately decreasing urate secretion from tubular cells **(Åkesson et al., 2014; Ljubojević et al., 2016).** Cadmium toxicity can disrupt the basolateral membranes of proximal tubules, impairing p-aminohippurate excretion and leading to impaired urate excretion and hyperuricemia **(Sun et al., 2017).**

Treatment with NSO led to decreased urea, uric acid, and creatinine concentrations and restored kidney weight, indicating improved renal function. Similar results were corroborated by Azzaz et al. **(Azzaz et al., 2022),** who administered rats with a solution comprising 2.4 g of $CdSO_4$ and 1.5 mL of NSO dissolved in 300 mL of distilled water at a dosage of 0.55 mL for 15 days. **Abdul Hannan et al. (2021)**

reported that the nephroprotective effect of NSO could be attributed to TQ's antioxidant and anti-apoptotic properties, which may be helpful for optimal effects. TQ had a regulatory effect on rat kidneys from heavy metal-induced injury by increasing SOD and GSH levels and inducing CAT, glutathione peroxidase, and glutathione reductase activities. **(Mabrouk and Cheikh, 2016).**

Oxidative stress is a pivotal molecular mechanism in chronic Cd exposure-induced nephrotoxicity **(Nemmiche, 2017).** In our results, two months of Cd exposure leads to increased protein carbonyls and MDA levels and decreased nonenzymatic and enzymatic antioxidants in rat liver and kidney. **Amamou et al. (2015)** revealed comparable outcomes; It has been suggested that depletion of GSH or suppression of antioxidant enzymes is responsible for increased lipid peroxidation by Cd, which binds strongly to GSH's -SH group, leading to its oxidation and accumulation as oxidized glutathione.

Anjum et al. (2024) indicate that the mechanism causing cadmium damage to the liver and kidney depends on generating reactive oxygen species, which modify normal cell function by interfering with mitochondrial activity and genetic information. However, this oxidative injury is corrected in rats receiving NSO in their diet. The protective effect of NSO may be attributed to TQ's antioxidant properties, acting as a lipid peroxidation inhibitor, which exhibits a comparable potential to that of TBHP and butylated hydroxytoluene, two commonly used synthetic antioxidants **(Nagi et al., 1999)**. **Zafeer et al. (2012)** found that pretreatment with TQ (10 μM) protected Swiss albino mice liver cells from protein oxidation and depletion of cellular antioxidants caused by $CdCl_2$ (5 mM) under in vitro conditions. This suggests that TQ has the potential to prevent oxidative stress-related liver diseases.

In addition, the analysis of NSO highlighted the presence of considerable quantities of phenolic compounds that exhibit potent antioxidant effects, such as gallic acid, with amounts ranging from 1.02 to 1.40 mg equivalents/g oil and a trace amount of dithymoquinone which contribute to human health **(Kiralan et al., 2021).** Gallic acid, a ubiquitous plant metabolite, features the chemical configuration of trihydroxybenzoic acid, characterized by numerous hydrogen atoms within its phenolic structure conducive to the facile delocalization of free radicals. Its capacity to mitigate inflammatory processes stems from its ability to counteract superoxide anions, impede the release and function of myeloperoxidase, and conceivably modulate the accumulation of active NADPH-oxidase **(Ojo et al., 2023).**

NSO also contains β-tocotrienol at 1195 mg/kg and the γ-tocopherol isomer at 208 mg/kg. Furthermore, β-sitosterol emerges as the predominant sterol compound within the unsaponifiable fraction, with concentrations ranging from 1135 to 1182 μg/g of oil, as reported by **Kiralan et al. (2021)**. Furthermore, α-tocopherol is a lipid-soluble antioxidant integrated into cell membranes to inhibit lipid peroxidation. It also acts as a scavenger of free radicals and can impede the activation of monocytes or other cytokine-secreting cells. Research indicates that β-tocotrienol exhibits superior potency and efficacy compared to α-tocopherol in safeguarding bone against oxidative stress toxicity **(Zarkasi et al., 2020).**

On the other hand, various studies have shown that mitochondria are a preferred target for Cd toxicity. The latter can alter enzyme function and membrane permeability and damage the mitochondrial structure by generating oxidative stress **(Wu et al., 2015; Korotkov, 2023).**

Our results showed an increase in the production of lipid and proteins peroxidation at the liver and kidney levels. This production of free radicals following exposure to Cd has been highlighted by many studies **(Wang et al., 2014; Pan et al., 2018).**

However, no significant difference in SOD activity was observed in Cd-intoxicated rats, which is consistent with the work of **Jihen et al. (2011)** and **Claudio et al. (2016).**

Under physiological conditions, there is a balance between the activity of the SOD (the enzyme responsible for the dismutation of O^{-}_{2} into H_2O_2) and the activity of the GPx (the enzyme that metabolizes H_2O_2). Thus, an increase in the concentration of H_2O_2 may suggest an imbalance between the activity of GPx and SOD in these organs. H_2O_2can accumulate in the mitochondria if the activity of GPx is lower than that of Mn-SOD, which explains the increased levels of H_2O_2 and the decrease in the concentration of GSH in the mitocondrial fraction in the liver and kidneys of rats intoxicated by CdCl2 **(Owen et al., 2010).**

The use of NSO significantly improved the redox state of the liver and kidney mitochondria, decreasing the level of prooxidants and increasing antioxidant activity. This confirms the effect of NSO in the prevention of the toxicity of Cd .

5. Conclusion

In conclusion, our study suggests that the consumption of NSO could prevent decreased body weight induced by Cd toxicity and restore liver function, as evidenced by the normalization of AST and ALT levels. Additionally, NSO appears to play a role in regulating urea, creatinine, and uric acid levels in the bloodstream, indicating potential benefits for kidney function and waste elimination. Furthermore, NSO demonstrates significant potential in reducing oxidative stress markers in both liver and kidney tissues. These findings suggest that NSO holds promise a potential utility as a dietary supplement to mitigate the harmful effects of Cd toxicity. Further research is warranted to elucidate its mechanisms of action and assess its efficacy in clinical settings.

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Credit authorship contribution statement

Radjaa Kaouthar Meziane: Methodology, Performed the experiments, Data curation, and Writing – original draft. **Amamou Fouzia**: Methodology, Performed the experiments, Data curation. **Didi Amel**: Software, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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