Original Research Article ISSN (Print) 2786-0272 ISSN (Online) 2786-0280

Efficacy of Olive Oil In Amelioration of Nephrotoxicity In Experimental Male Rats

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Received at: 2023-08-06 Accepted at: 2023-08-29

ABSTRACT: The kidney is an essential part of the urinary system responsible for hormone secretion and blood filtration from waste products. Nephrotoxicity (NEP) is one of the most popular kidney diseases and originates from environmental toxic agents and a wide spectrum of therapeutic drugs. Olive oil (OO) is among the natural oils extracted from olives. It offers many health benefits against chronic diseases and has an immunostimulant role. The study was designed to predict the reno-protective impact of OO opposing NEP. The research model was performed using three groups of adult male albino rats (n=30); the control group, NEP induced group by carbon tetrachloride, and the third group protected against NEP by OO. Kidney function was evaluated by measuring serum urea, creatinine, uric acid, and electrolytes. Further, the oxidative stress parameters, thiobarbituric acid reactive substances, nitric oxide, and reduced glutathione were analyzed in renal tissue homogenate. In addition, the ELISA technique assessed both tumor necrosis factor-alpha and interleukin-6. The histopathological study of renal tissue was also inspected. The results demonstrated that OO has an excellent protective effect, such as greatly restoring the higher level of kidney parameters and oxidative stress injury. Further, OO declined the induced inflammation and fibrosis in the protective group compared to NEP. Therefore, due to OO promising nephroprotective potentiality, it could be used to avoid disorders linked to oxidative stress. It is strongly recommended to conduct further research on its application.

KEYWORDS: Kidney; Nephrotoxicity; Olive oil; Reactive oxygen species; Toxic agents.

1. Introduction

The kidney is one of the essential body organs in conserving the homeostasis and detoxifying process [1]. Kidney disorders (KD) are a serious global public health issue. Both acute and chronic kidney diseases are considered KD types [2]. Acute kidney disease (AKD) usually originates from old age and some chemotherapeutic and nephrotoxic drugs [3]. However, chronic kidney disease mainly results from untreated AKD or complications from other chronic diseases, such as diabetes mellitus [4]. Nephrotoxicity (NEP) is a syndrome that occurs due to the toxic effect of chemicals and overdoses of drugs and leads to failure of the kidney to perform its normal function [5]. It was reported that NEP could be induced by exposure to overdosage of some therapies such as paracetamol [6], heavy metals like cadmium [7] and chemotherapeutic agents such as doxorubicin and cisplatin [8]. Natural sources of antioxidants have a beneficial role in the protection of some diseases. Olive oil (OO) is a vegetable oil produced from the cultivated forms of the *Olea europaea* plant, which is a member of the Oleaceae family, and it is one of the key dietary components that have a unique health benefit [9]. According to the extraction method of OO, there are various types, including extra virgin OO, virgin OO, refined OO, and pomace OO [10]. The chemical composition of OO involves monounsaturated and polyunsaturated fatty acids [11], diacylglycerol, and color pigments such as carotenoids and chlorophyll [12]. OO efficiently produces numerous wastes and byproducts [13]. OO displays a broad spectrum of health benefits. It has antithrombotic [14], antimicrobial [15], antiapoptotic [16], and antiallergic [17] properties. Also, it has neuroprotective [18], antidiabetic and cardioprotective effects [19]. The current study's purpose is to inquire about the influence of OO on NEP-induced oxidative stress and inflammation.

2. Materials and methods

2.1. Chemicals, reagents, and kits

5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), carbon tetrachloride (CCL_4), reduced glutathione (GSH), and thiobarbituric acid (TBA) were derived from Sigma-Aldrich, USA. Hematoxylin and Eosin (H and E) stain, N-naphthyl ethylenediamine dihydrochloride, sulfanilamide, trichloroacetic acid, and xylene were obtained from Ricca Chemical Company (Arlington), Coinbrook Bucks (England), Win Lab, LOBA-Chemie and Taiwan, respectively. Absolute ethyl alcohol, disodium hydrogen phosphate, formalin, phosphoric acid, sodium dihydrogen phosphate, and sodium chloride were got from El-Nasr Pharmaceutical Chemical Company, Egypt. Also, sodium nitrite and sodium nitroprusside were coming from Merck company. Isoflurane 1% was bought from Hospira Inc (USA) and OO from Roth (Australia). Moreover, urea, uric acid, sodium (Na^+), potassium (K^+), and chloride (Cl^-) kits were got from Spectrum, Egypt. Creatinine fixed rate kits were acquired from Biomed, Egypt. Rat tumor necrosis factor-alpha (TNF-α) and interleukin-6 (Il-6) ELISA kits were purchased from Elabscience Biotechnology (INC).

2.2. Animals

Albino rats (30 adult males) weighing 120-160 g used for this work were purchased from the animal house, Faculty of Medicine, Assuit University, Egypt. The study was performed under the approved ethical regulation of the Institutional Review Board, Faculty of Medicine, Assiut University, 04-2023-200253. Rats were kept in cages with unlimited food and water (H_2O) access, and they were kept under observation for adaptation under standardized laboratory circumstances.

2.3. Experimental induction of NEP and protection approach

The rats were acclimatized before being classified into three groups (10 rats/group) as follows: The first group was normal rats (NR), receiving only distilled H_2O every day via an intra-gastric tube for one month. The second group was NEP induced group, where the rats were orally administrated 0.5 ml/kg Bwt CCL_4 dissolved in OO in a ratio of 1:3 twice a week for a month. The selected dose of CCL_4 is based on previous publications with slight modifications [20]. The last group was protected group against the NEP by OO (NEP-OO), where they were orally administered 1 ml OO/kg Bwt daily for one month [21] together with 0.5 ml/kg Bwt CCL_4 twice a week during the same month. At the end of the fourth week, the rats fasted for 12 h with free access to H_2O, then anesthetized with isoflurane, according to [22]. After scarification, blood samples and kidney tissue were collected under appropriate laboratory standards. Serum was separated by centrifugation at 3000 rpm for 15 min. and stored at -80°C. Also, the left kidney was used for homogenate preparation in ice-cold phosphate buffer saline (PBS), 0.1 M, pH 7.4, and stored at -80°C for biochemical assays. Moreover, the right kidney was stored for histopathological analysis in a 10% neutral buffered formalin.

2.4. Assessment of kidney function parameters

Indicators of kidney function were estimated according to the kit's instructions. Both serum urea and uric acid levels were analyzed by photometric kits, while kinetic kits assessed creatinine concentration. Further serum Na^+, K^+, and Cl^- were evaluated by colorimetric kits.

2.5. Determination of thiobarbituric acid reactive substances (TBARS)

The quantity of TBARS was estimated in renal tissue by measuring the reaction of malondialdehyde as a major secondary product of lipid peroxidation with TBA to yield a pink-colored product, which was measured at 540 nm via spectrophotometer (JENWAY 61431, 6705, UK) [23].

2.6. Estimation of nitric oxide (NO)

The NO amount in renal tissue was analyzed in line with [24]. Briefly, 150 µL of 10 mM sodium nitroprusside in 0.1 M PBS, pH 7.6, was combined with 100 µL of renal homogenate supernatant, and the mixture was incubated for 2 hours in the dark at room temperature. Following that, 250 µL of *Griess* reagent (10 mM sulfanilamide and 1 mM N-naphthyl ethylenediamine dihydrochloride in 5%

phosphoric acid) was incorporated. The absorbance of samples and reference (1 mg/ml sodium nitrite in phosphoric acid 5%) was measured at 540 nm against the blank and stated as nM/mg protein.

2.7. Estimation of GSH

Assessing GSH in renal tissue samples is vital to monitor the redox process inside the cell [25]. The test depended on the reaction of GSH with DTNB in the existence of glutathione reductase enzyme creating a yellow-colored 2-nitro-5-thiobenzoic acid product that was measured via spectrophotometer at 412 nm [26].

2.8. Inflammatory biomarkers

The TNF-α and Il-6 assays were carried out using the manufacturer's guidelines by the quantitative sandwich enzyme immunoassay method. TNF-α and Il-6-specific antibodies have been pre-coated onto ELISA microplates. The standard or renal tissue homogenate sample combined with the antibody of interest. Enzymatic processes produced a blue substance that changed yellow when introducing the stop solution. The absorbance was read at 450 nm using an ELISA reader (HEEPF D-080-HO, Biotec, USA). Using a standard curve, the levels of TNF-α and Il-6 in the renal tissue homogenate were calculated in pg/mg tissue protein.

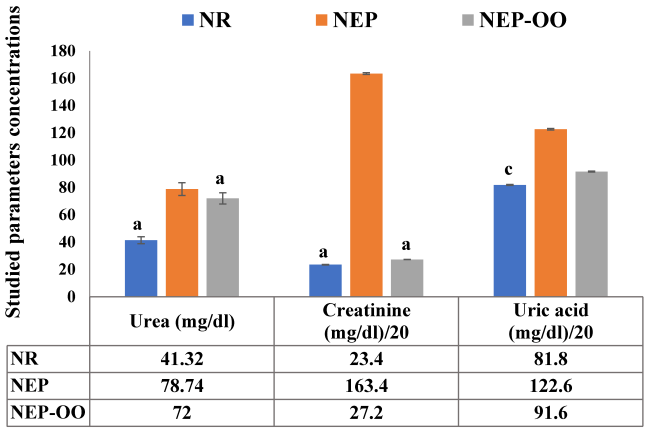
2.9. Histopathological evaluation of renal tissue

The histopathological examination of renal tissue was carried out. After scarification, the right kidney was removed from the studied rat groups and fixed in a 10% neutral buffered formalin for 24 hrs. After that, the dehydration phase was performed in ascending rates of ethyl alcohol. The tissues were put in xylene, implanted in paraffin wax, then exposed to H and E stains. Finally, the tissue sections between three and five microns thick were examined by a light microscope.

2.10. Statistical analysis

Statistical analysis was accomplished by the SPSS program (Version 25, One way ANOVA). Data are presented as mean ± SE, and the p-value ≤ 0.05 was statistically significant.

3. Results

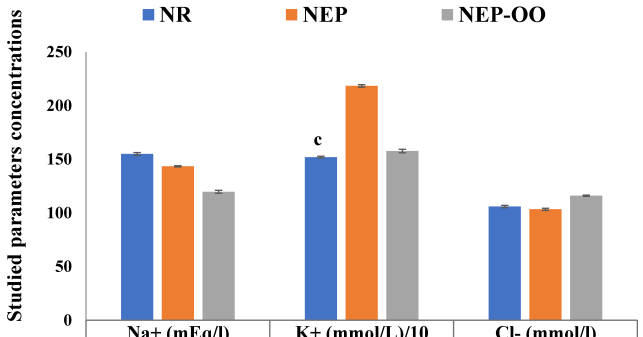


	Urea (mg/dl)	Creatinine (mg/dl)/20	Uric acid (mg/dl)/20
NR	41.32	23.4	81.8
NEP	78.74	163.4	122.6
NEP-OO	72	27.2	91.6

Figure 1: Serum urea, creatinine, and uric acid concentrations of NR, NEP, and NEP-OO groups. Values are expressed as mean ± SE (n=10). Significance: $^{a}P \leq 0.001$, $^{b}P \leq 0.01$, $^{c}P \leq 0.05$ compared to NEP

3.1. The outcome of OO on serum renal performance

Serum urea declined by 47.52% and 8.56% in NR and NEP-OO groups, respectively, compared to the NEP group. Further, when comparing data of creatinine concentration in NR and NEP-OO groups by NEP group, it was noticed reduction by 85.68% and 83.35%, respectively. Moreover, uric acid dropped by 33.28% compared to the NEP group. Despite there being a reduction in uric acid in the protected group by OO in comparison to the NEP group, this reduction was non-significant ($p \leq 0.05$) (Fig. 1).

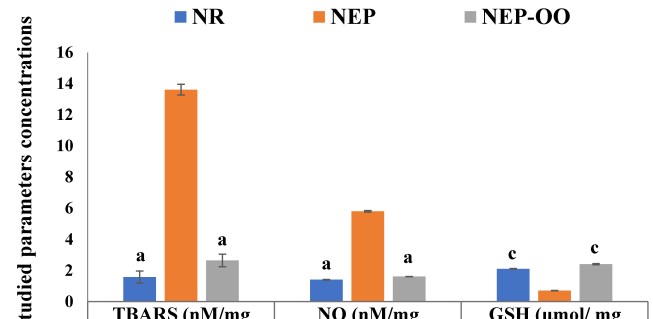


	Na+ (mEq/l)	K+ (mmol/L)/10	Cl- (mmol/l)
NR	154.89	151.9	105.94
NEP	143.42	218.3	103.32
NEP-OO	119.68	157.6	116.02

Figure 2: Serum Na^+, K^+, and Cl^- concentrations of NR, NEP, and NEP-OO groups. Values are expressed as mean ± SE (n=10). Significance:$^{a}P \leq 0.001$,$^{b}P \leq 0.01$,$^{c}P \leq 0.05$ compared to NEP

As opposed to that, NEP didn't cause an increase or decrease in Na^+ and Cl^- concentration. But K^+ level was

raised by 30.42% in NEP compared to the NR group, and such an increase couldn't be restored during OO's protective strategy (Fig. 2).



	TBARS (nM/mg protein)	NO (nM/mg protein)/10	GSH (µmol/ mg protein)/10
NR	1.57	1.4	2.1
NEP	13.61	5.8	0.7
NEP-OO	2.64	1.6	2.4

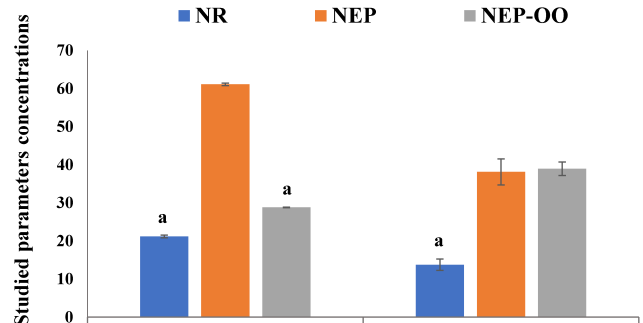
Figure 3: Renal TBARS, NO, and GSH concentrations of NR, NEP, and NEP-OO groups. Values are expressed as mean ± SE (n=10). Significance:$^{a}P \leq 0.001$,$^{b}P \leq 0.01$,$^{c}P \leq 0.05$ compared to NEP.

3.2. *Effect of OO on renal cellular redox and inflammatory status*

Results in Fig. 3 revealed a remarkable elevation of TBARS and NO concentrations by 88.46% and 75.86% in the NEP group compared to NR. Using OO as a protective therapy against NEP exhibited a reduction of elevated TBARS by 80.60% and NO by 72.41%. Also, the GSH content of the NEP group decreased nearly 2-fold compared to NR and increased in the NEP-OO group by 2.43-folds. The rats affected by NEP displayed higher TNF-α and Il-6 levels by 65.30% and 63.93%, respectively, compared to NR. Renal TNF-α declined by 52.86% in the NEP-OO group compared to the NEP group. However, Il-6 wasn't corrected in the NEP-OO group ($p \leq 0.05$) (Fig. 4).

3.3. *Histopathological results of the renal medulla*

The renal medulla was examined against pathological abnormalities in all studied groups. NR showed normal medullary tubules with a normal and clear lumina (Black arrow) (Fig. 5a). At the same time, NEP exposed dilated tubules with flattened uroepithelium and complete degeneration (Black arrow), and severe fibrosis (Red arrow) Fig. 5b. The protected group by OO showed moderate



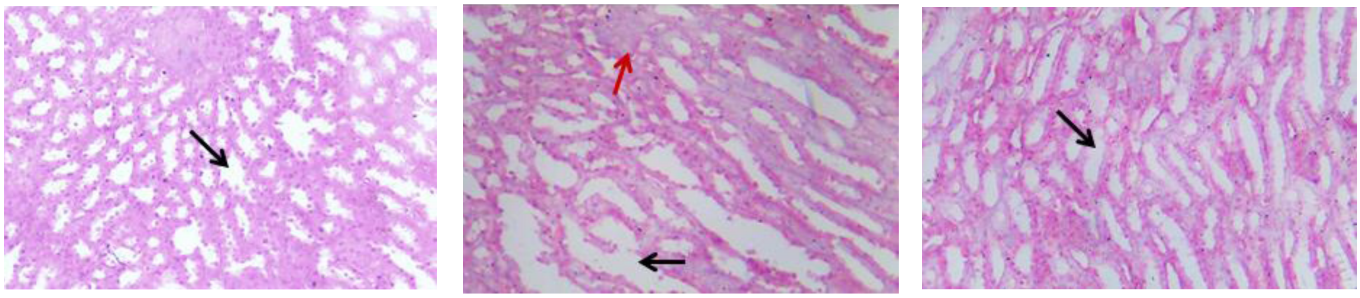
	TNF-α (pg/mg protein)/10	Il-6 (pg/mg protein)
NR	21.2	13.75
NEP	61.1	38.12
NEP-OO	28.8	38.94

Figure 4: Renal TNF-α and Il-6 concentrations of NR, NEP, and NEP-OO groups. Values are expressed as mean ± SE (n=10). Significance:$^{a}P \leq 0.001$,$^{b}P \leq 0.01$,$^{c}P \leq 0.05$ compared to NEP

regeneration of dilated tubules and flattened uroepithelium (Black arrow) (Fig. 5c).

4. Discussion

Oxidative stress (OS) is one of the processes causing cellular death in various diseases [27]. The kidney is the most crucial excretory organ, where all toxic metabolites are eliminated via urine. The toxic impact of CCL_4 on kidney function is mainly accredited to the OS caused by a high level of reactive oxygen species generation [28]. Both the liver and kidney are involved in CCL_4 metabolic pathway. In the kidney, CCL_4 metabolism is performed inside the endoplasmic reticulum of the proximal epithelial tubule by cytochrome P4502E1 enzyme to the trichloromethyl ($CCL_3^{\cdot}$) radicals [29]. The formed $CCL_3^{\cdot}$ is oxygenated to highly reactive trichloromethyl peroxyl ($CCL_3OO^{\cdot}$). Both $CCL_3^{\cdot}$ and $CCL_3OO^{\cdot}$ cause the destruction of kidney cells and organelle membranes by promoting extracellular matrix (ECM) synthesis and deposition, which is included various protein complexes, such as elastin, collagen, glycoproteins, and proteoglycan[30]. The administration of CCL_4 led to an increase in urea, creatinine, and uric acid concentrations, which indicates acute renal failure with proximal tubule harm [31], and these results were in accordance with other studies [32]. From the biochemical background, the elevated creatinine level is ascribed to deficient glomerular filtration due to loss in glomeruli function. Also, the significant rise in the urea and uric acid



(a) NR **(b)** NEP **(c)** NEP-OO

Figure 5: Renal medulla tissue sections microscopical images in different rat groups stained with H & E

concentrations was due to improved protein breakdown and, afterward, enhanced urea creation. The data in the existing study showed that elevated K^+ concentration in the CCL_4 group correlated to NEP; however, both Na^+ and Cl^- were at the normal level, and these results disagreed with [33, 34] who reported an elevated level of both Na^+ and K^+ in CCL_4 induced renal injury in the experimental rat model. Incidence of NEP by CCL_4 leads to a higher level of both TBARS and NO in the renal tissue, and that was linked to the acidic media of CCL_4 that destruct the renal vascular endothelium and enhance the process of lipid peroxidation and biosynthesis of NO with huge amount [35]. The current information was concurred with [36, 37]. At the same time, our results revealed a reduction of the GSH concentration, which ascribed to the accumulation of hydrogen peroxide and toxic metabolites produced from CCL_4 [38] and this was in agreement with [39]. Also, the poisonous effect of $CCL_3^{\cdot}$ and $CCL_3OO^{\cdot}$ metabolites on the proximal nephrocytes causes stimulation of nuclear factor-kappa-B and leukocytes which leads to the elevation of a pro-inflammatory cytokine, TNF-α [40]. Upregulation of TNF-α and Il-6- was reported in other previous research [41, 42]. Consumption of OO in Mediterranean food occupies a vital role in diminishing the risk of some diseases. The main monounsaturated fatty acids (MUFAs) in OO are the oleic acid that forms 80% of its composition, palmitic acid, and linoleic acid [43]. Those MUFAs are characterized by their ability to resist the OS because the presence of only one double linkage in their chemical structure [44]. The chief phenolic substances in OO are oleuropein, hydroxytyrosol, and tyrosol, which support it by the highest antioxidant and radicals scavenging potency due to their ability to donate hydrogen and enhance the radical stability [45]. Our research revealed that OO has a protective influence against elevated TBARS and NO and the reduction of GSH induced in NEP, which matched [46] results. Further, the observation of [47] showed that OO hinders the deposition of ECM in the heart of the offspring of diabetic rats and hence reduces the elevated TBARS and NO levels. Additionally, unsaturated fatty acids in OO regulate inflammatory markers [48]. The current data evoked that OO restored both TNF-α and Il-6 in the NEP-OO group, which comes in accordance with [49]. In this study, we also investigated that serum urea and creatinine were decreased in protected rats with OO prior to their exposure to NEP, which was in harmony with [50]. In line with these biochemical discoveries, the histopathological study of the kidney tissue discovered that NEP induced numerous modifications in the renal medulla; however, those lesions declined in the NEP-OO group.

Conclusion

The present work has shown the nephrotoxic influence of carbon tetrachloride (CCL_4) on kidney dysfunction via alteration of the prooxidant and antioxidant cellular status and some inflammatory markers. The olive oil (OO) had a positive protective impact against the induced toxicity by CCL_4 in male rats at biochemical and histopathological levels. In spite of that, more investigation is required to explore the toxic effects of CCL_4 on various body organs and the protecting action of OO compared with other natural antioxidants.

Acknowledgments

The authors thank all participants for their support during this work.

Conflict of interest statement

The authors haven't a conflict of interest to declare

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