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Original Article

Effect of oleuropein against chemotherapy drug-induced histological changes, oxidative stress, and DNA damages in rat kidney injury

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ABSTRACT

Cisplatin-based chemotherapy is responsible for a large number of renal failures, and it is still associated with high rates of mortality today. Oleuropein (OLE) presents a plethora of pharmacological beneficial properties. In this study we investigated whether OLE could provide sufficient protection against cisplatin-induced nephrotoxicity. With this aim, Sprague-Dawley rats were divided into eight groups: control; 7 mg/kg/d cisplatin, 50 mg/kg, 100 mg/kg, and 200 mg/kg OLE; and treatment with OLE for 3 days starting at 24 hours following cisplatin injection. After exposure to the chemotherapy agent and OLE, oxidative DNA damage was quantitated in the renal tissue of experimental animals by measuring the amount of 8-hydroxy-2′-deoxyguanosine (8-OHdG) adducts. Malondialdehyde (MDA) level, total oxidative stress (TOS), and total antioxidant status (TAS) were assessed to determine the oxidative injury in kidney cells. The histology of the kidney was examined using four different staining methods: hematoxylin-eosin (H&E), periodic acid Schiff (PAS), Masson trichrome, and amyloid. In addition, the blood urea nitrogen (BUN), uric acid (UA), and creatinine (CRE) levels were established. Our experimental data showed that tissue 8-OHdG levels were significantly higher in the cisplatin group when compared to the control group. The glomerular cells were sensitive to cisplatin as tubular cells. In addition, treatment with cisplatin elevated the levels of BUN, UA, CRE, and TOS, but lowered the level of TAS compared to the control group. The OLE therapy modulated oxidative stress in order to restore normal kidney function and reduced the formation of 8-OHdG induced by cisplatin. Furthermore, the OLE treatment significantly reduced pathological findings in renal tissue. We demonstrate for the first time that OLE presents significant cytoprotective properties against cisplatin-induced genotoxicity by restoring the antioxidant system of the renal

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tissue. According to our findings, OLE is a promising novel natural source for the prevention of serious kidney damage in current chemotherapies.

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1. Introduction

The efficiency of chemotherapy is critical for its success. Cisplatin is one of the most potent chemotherapeutic drugs acting against various solid tumors; however, its use is mainly limited due to the emergence of nephrotoxicity, neurotoxicity, myelotoxicity, and ototoxicity [1]. Cisplatin is primarily excreted renally. Cisplatin-associated severe acute kidney injury occurs in 20% of patients and has a negative impact on long-term renal function and patient survival [2]. Cisplatin-induced renal injury is progressive and can be severe and irreversible. Cisplatin can directly induce necrosis and apoptosis of renal tubular cells. Thus, impaired kidney function may result in long-term complications for patients [3,4]. In addition, cisplatin-induced urine electrolyte wasting contributes to hypocalcemia and hypokalemia [5]. Furthermore, cisplatin treatment increases oxidative stress, inflammation, and also fibrinogenesis [6,7].

Although great advances in cancer treatments such as chemotherapy, surgery, and radiation are currently being achieved, their application is associated with numerous and expensive adverse side effects [8]. To the best of our knowledge, the pharmacology underlying renal injury induced by cisplatin is well documented; however, the best drug to prevent it remains undefined. To conserve renal units in response to a decline in renal function, natural sources have become the alternative therapy for early kidney damage. This new concept has deeply affected the therapeutic area. The alternative drugs have been investigated in order to minimize serious side effects of cisplatin and to enhance its antineoplastic efficacy [9]. Indeed, many synthetic compounds are highly toxic and exert side effects even though they are effective in inhibiting cancer cell growth. Using natural compounds or their derivatives is one way to solve these problems [10]. Due to its established beneficial effects on health, olive leaf has gained the interest of the scientific and industrial community. Olive leaf components have antimicrobial, antioxidant, antiviral, antihypertensive, anti-inflammatory, hypoglycemic, and neuroprotective properties. Additionally, these components are used in cosmetic products [11]. The predominant natural constituent of the leaves of the olive tree is oleuropein (OLE), which presents a plethora of beneficial pharmacological properties [12,13].

OLE is an inexpensive and readily available phenolic compound. To achieve a low cost and effective treatment, the current study aimed to investigate the effectiveness of OLE on the restoration of the cisplatin-induced nephrotoxicity in rats. There are many techniques with the ability to identify and measure cellular DNA damage upon exposure to a suspected genotoxic agent; however, the modified nucleoside 8-hydroxy-2′-deoxyguanosine (8-OHdG) is commonly used as a reliable and sensitive index of oxidative DNA damage [14]. Therefore, in this study, we examined oxidative DNA damage in renal cells of rats with cisplatin-induced kidney damage by measuring the levels of 8-OHdG. In addition to this oxidative DNA analysis, we have measured the sensitive metabolic biomarkers in the blood, the oxidative alterations and histological evaluation of rat kidney tissues

2. Material and methods

2.1. Animals

Fifty-six adult male Sprague-Dawley rats (weighing 200–250 g) obtained from the Medical Experimental Application and Research Center, Atatürk University, Turkey, were used. Animals were housed inside polycarbonate cages in an air-conditioned room (22°C ± 2°C) with 12-hour light-dark cycle. Standard rat feed and water were provided *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for 7 days before the start of the experiment. This study was carried out in the Experimental Animals Research Centre of Atatürk University. The experiments were approved by the Local Ethics Committee for Experiments on Animals of Atatürk University (protocol number: B.30.2.ATA.0.23.85-11). All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources Commission on Life Sciences [15].

2.2. Chemicals

Both cisplatin and OLE [high performance liquid chromatography (HPLC) grade ≥ 98%] were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). In order to determine 8-OH-dG levels in the kidney samples, 8-hydroxy-2′-deoxyguanosine assay kits were used (Cayman Chemical Co., Ann Arbor, MI, USA). All drug and chemicals were freshly prepared before each administration. The doses of cisplatin and OLE used in this study were selected according to our preliminary experiments and in accordance with previous reports [16,17].

2.3. Experimental study design

The rats were weighed (250 g) and randomly allocated into eight experimental groups ($n = 7$). In Group 1 (control), the animals received 1 mL of distilled water as vehicle. In Group 2 (cisplatin), the animals received 7 mg/kg/d, diluted in distillated water (1 mL). In Groups 3, 4, and 5 (OLE), rats received 1 mL of OLE solution (50 mg/kg/d, 100 mg/kg/d, and 200 mg/kg/

d, respectively). In Groups 6, 7 and 8 (Cisplatin/OLE), the animals received 1 mL of OLE solution (50 mg/kg/d, 100 mg/kg/d, and 200 mg/kg/d, respectively) following cisplatin administration.

The injections of cisplatin were given using a single intraperitoneal (ip) dose for 24 hours. The OLE and Cisplatin/OLE groups received ip injections with a daily single dose for a total period of 3 days. On Day 4 after injections, the rats were anesthetized with isoflurane and blood samples were collected for biochemical studies. After cervical dislocation under anesthesia, the kidney specimens were gathered for further analyses.

2.4. *Biochemical analysis*

2.4.1. *Preparation of renal tissue homogenates*

Fresh kidney tissues were rinsed with ice-cold saline and immediately stored at −80°C. The tissue specimens were weighed and then homogenized in a 50mM phosphate buffered saline at pH 7.0. Homogenized kidney tissues were then centrifuged at 12,298 g at 4°C for 15 minutes to isolate the supernatant for subsequent analysis.

2.4.2. *Measurement of nucleic acid oxidation*

The 8-hydroxy-2′-deoxyguanosine assay is used for the quantification of 8-OHdG in homogenates and recognizes both free 8-OH-dG and DNA-incorporated 8-OH-dG. This assay depends on the competition between 8-OHdG and 8-OHdG-acetylcholinesterase (AChE) conjugate (8-OH-dGTracer) for a limited amount of 8-OHdG monoclonal antibody which has been commonly used in many studies. All procedures were carried out in accordance with the provider manual. DNA was digested by incubation with DNase I, endonuclease, and alkaline phosphatase [18]. The amount of 8-OHdG was measured by HPLC with electrochemical detection.

2.4.3. *Measurement of malondialdehyde*

Lipid peroxidation was determined by quantifying the malondialdehyde (MDA) content in the tissue supernatant. Thiobarbituric acid (TBA, 0.8% w/v) was added to the reaction mixture after vortexing the contents of the tube. The tubes were kept in a boiling water bath for 1 hour. The MDA level was determined by the absorption coefficient of the MDA–TBA complex at 532 nm using a spectrophotometer [19].

2.4.4. *Measurement of tissue total antioxidant status and total oxidant status levels*

To evaluate total antioxidant status (TAS) in the kidney, tissue homogenate was added to a mixture of sodium phosphate buffer (100 mmol/L, pH 7.4), sodium benzoate (10 mmol/L). Then, acetic acid, freshly prepared Fe–EDTA and H_2O_2 (10 mmol/L) were added and incubated for 60 minutes at 37°C. Acetic acid and TBA were added and incubated for 10 minutes at 100°C (in a boiling water bath) then cooled in an ice bath. TBA reactive substance was measured by spectrophotometer at 532 nm [20].

Total oxidant status (TOS) from rat kidneys was measured via colorimetric methods by using commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey). Oxidants present in the sample oxidize the ferrous ion-O-dianisidine complex to the ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide, and TOS units were expressed in terms of mmol H_2O_2 equivalent per gram of tissue. An indicator of the degree of oxidative stress was calculated according to the following formula: oxidative stress = TOS/TAS [21].

2.4.5. *Measurement of blood urea nitrogen, creatinine, and uric acid levels*

Blood urea nitrogen (BUN), creatinine (CRE), and uric acid (UA) levels were measured based on an enzymatic method using a Beckman Coulter AU680 analyzer (Beckman Coulter, Miami, FL, USA).

2.5. *Histological examinations*

Renal tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned (5 μm), and placed on slides. The samples were stained with hematoxylin and eosin (H&E) for identification of architectural changes. Periodic acid Schiff (PAS) and amyloid staining methods were used to identify glycogen content and proteinaceous casts in the kidney, respectively. Additionally, Masson trichrome staining method was used to evaluate the renal fibrosis. The high-resolution pictures of samples (200×) were taken under bright field using an Olympus BX60 microscope (Olympus, Hamburg, Germany).

2.6. *Histological assessments*

Kidney histological assessment was determined by congestion, inflammation, tubular apoptosis, tubular dilatations, amyloid cast, interstitial fibrosis, and glomerular damages (glomerular fibrosis and mesangial matrix expansion). The pathologic slides were scored on a scale of 0–4 according to the severity of renal damage using a scoring system previously described by Jablonski et al [22], Teixeira et al [23] and Rule et al [24].

2.7. *Statistical analysis*

For statistical analysis, we used SPSS for Windows 18.0 (SPSS Inc., Chicago, USA). The experimental data were analyzed using one-way analysis of variance followed by Turkey post hoc test for multiple comparisons. Results are presented as mean ± standard error (SE) and values of $p < 0.05$ were regarded as statistically significant.

3. Results

As presented in Table 1, the 8-OHdG levels, a hallmark of oxidative stress-DNA base damage, were markedly increased in rat kidney after cisplatin administration. On the contrary, there was no significant difference between the levels of 8-OHdG in the control and all OLE treated groups. Moreover,

Table 1 – The effects of OLE on renal 8-OH-dG levels after treatment with CIS.

Groups	8-OH-dG level (pg/mL)
Control	0.39 ± 0.16[a]
CIS	2.47 ± 1.26[d]
OLE 50 mg/kg	0.32 ± 0.11[a]
OLE 100 mg/kg	0.36 ± 0.12[a]
OLE 200 mg/kg	0.38 ± 0.16[a]
CIS + OLE 50 mg/kg	1.83 ± 0.83[b]
CIS + OLE 100 mg/kg	0.65 ± 0.14[c]
CIS + OLE 200 mg/kg	0.37 ± 0.18[a]

Data are presented as mean ± SD ($n = 7$).
The groups in the same column with different superscript letters are statistically significant ($p < 0.05$).
CIS = cisplatin; OLE = oleuropein; SD = standard deviation.

levels of 8-OHdG caused by cisplatin were normalized by a dose of 200 mg/kg OLE.

In the rat kidney tissues, the cisplatin group displayed remarkable elevation in the levels of MDA and TOS (Figures 1 and 2). Increases in serum BUN, CRE, and UA concentrations were also observed in the cisplatin group (Figures 3–5). However, TAS level was significantly reduced after cisplatin administration (Figure 6). The supplementation with OLE resulted in the reversal of biochemical parameters when compared with the cisplatin group. These effects were associated with the increasing dose of OLE therapy. Notably, all parameters reached statistical significance in the high-dose OLE group as compared to the group treated with cisplatin ($p < 0.05$).

3.1. *Histopathological findings*

In the control group, the normal architecture of tissue in sections stained with H&E (Figures 7A and 7B), PAS (Figure 7C), Masson trichrome (Figure 7D), and amyloid (Figure 7E) are shown. Examination of kidney sections in OLE groups

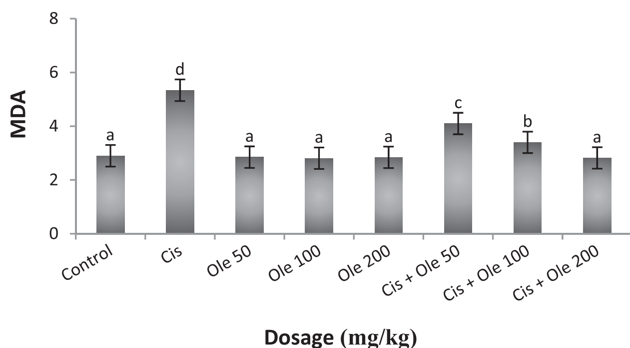


Figure 1 – The effects of OLE on kidney MDA level after treated with cisplatin. (Data are presented as mean ± SD (n = 7). Different letters indicate significant differences between studied groups at p < 0.05. CIS: cisplatin; MDA: malondialdehyde; OLE: oleuropein; SD: standard deviation.

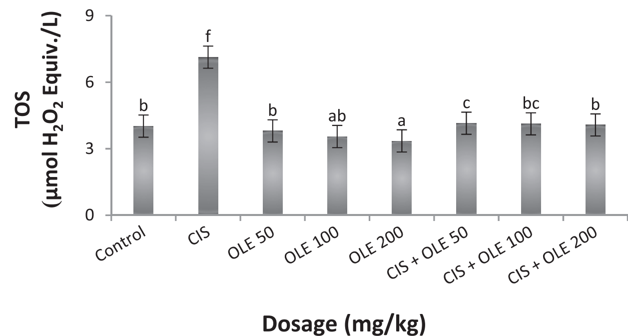


Figure 2 – The effects of OLE on kidney TOS level after treated with cisplatin. (Data are presented as mean ± SD (n = 7). Different letters indicate significant differences between studied groups at p < 0.05. CIS: cisplatin; OLE: oleuropein; TOS: total oxidative status; SD: standard deviation.

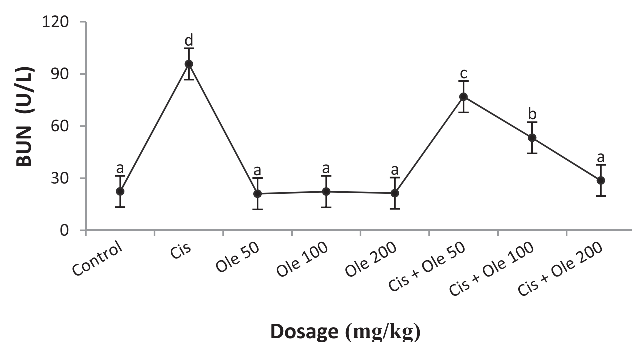


Figure 3 – The effects of OLE on serum BUN level after treated with cisplatin. (Data are presented as mean ± SD (n = 7). Different letters indicate significant differences between studied groups at p < 0.05. BUN: blood urea nitrogen; CIS: cisplatin; OLE: oleuropein; SD: standard deviation.

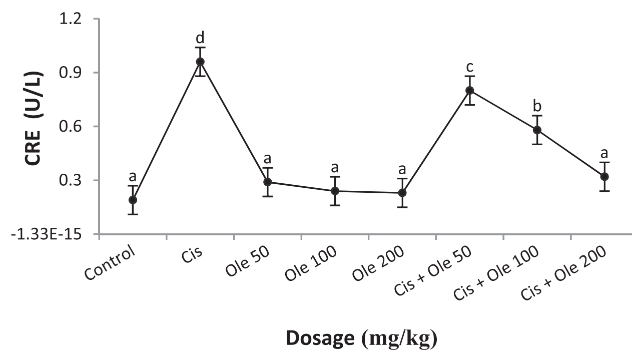


Figure 4 – The effects of OLE on serum CRE level after treated with cisplatin. (Data are presented as mean ± SD (n = 7). Different letters indicate significant differences between studied groups at p < 0.05. CIS: cisplatin; CRE: creatinine; OLE: oleuropein, SD: standard deviation.

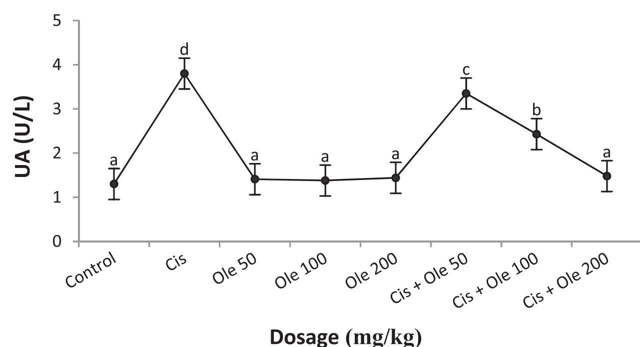


Figure 5 – The effects of OLE on serum Uric acid (UA) level after treated with cisplatin. (Data are presented as mean ± SD (n = 7). Different letters indicate significant differences between studied groups at p < 0.05. CIS: cisplatin; OLE: oleuropein; SD: standard deviation; UA: uric acid.

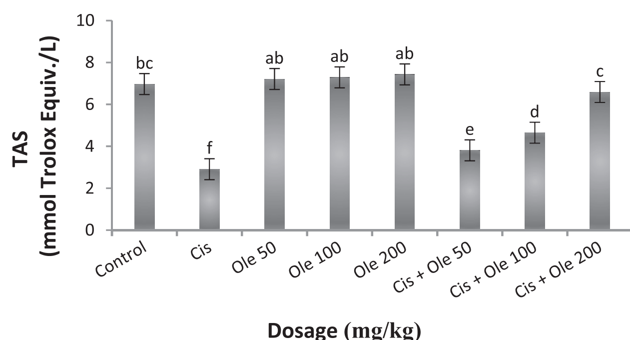


Figure 6 – The effects of OLE on kidney TAS level after treated with cisplatin. Data are presented as mean ± SD (n = 7). Different letters indicate significant differences between studied groups at p < 0.05. CIS: cisplatin; OLE: oleuropein; SD: standard deviation; TAS: total antioxidant status.

revealed that the kidney tissue retained its normal architecture (at 50 mg/kg, 100 mg/kg, and 200 mg/kg; data not shown).

An in-depth characterization of renal pathology samples stained by different methods is presented in Figure 8. In H&E stains, the microscopic observations showed significant congestion, infiltration, tissue necrosis with tubular degeneration and dilatations, amyloid cast, interstitial fibrosis and arteriosclerosis when compared with control rats. The mesangial proliferation in glomeruli was evident (Figures 8A–8E). PAS staining clearly revealed depletion of glycogen in renal cells and accumulation of amyloid compared with controls (Figure 8F). Masson trichrome (Figures 8G and 8H), and amyloid (Figure 8I) staining methods showed increased intensity of fibrosis in interstitial tissue and glomeruli and also dense amyloid inclusions indicating accumulation of protein, respectively.

In cisplatin+50 mg/kg OLE group, there was a reduction in congestion and mesenchymal proliferation in glomeruli (Figure 9A). After 100 mg/kg OLE treatment, tubular dilatations and degenerations were decreased (Figure 9B). Treatment with 100 mg/kg OLE for 3 days also increased the glycogen content of renal cells and decreased the intensity of fibrosis in the kidney of cisplatin groups (Figures 9C and 9D). A significant protective effect was observed after treatment with 200 mg/kg OLE. Kidney tissue showed a normal structure and orderly arrangement and resembled those of control rats (Figures 9E and 9F). Glomerular and tubulointerstitial lesions of the groups were scored in Table 2. The degree of pathological findings showed a significant difference between groups treated with cisplatin and cisplatin + OLE ($p < 0.05$).

4. Discussion

Cisplatin is the first Food and Drug Administration approved anticancer drug; however, the major limitation of cisplatin therapy is the development of nephrotoxicity, which may involve oxidative stress, inflammation, and cell death [25,26]. Nowadays, new drugs are tested to reduce side effects of chemotherapy. In this article, we demonstrate that OLE substantially reduces the genotoxicity of cisplatin in kidney cells. In fact, we found a significant improvement in cell damage in kidneys treated with OLE, compared to those treated with cisplatin. This protective effect seems to be related to the ability of OLE to prevent with formation of the reactive oxygen species (ROS) and to minimize renal dysfunction.

The cytotoxic effects of chemotherapy are mediated primarily through the generation of ROS and their by-products causing cell damage [27]. We chose MDA and 8-OHdG as candidates of important markers for intrarenal oxidative stress caused by cisplatin in this study, and evaluated their amounts in rat kidneys with cisplatin-induced acute renal failure. We showed that intrarenal MDA level was significantly increased following cisplatin injection in animals. The same results were revealed for TOS levels among rats treated by the chemotherapy drug. Oxidative damage can induce by lipid peroxidation depending on the increase of ROS [28]. MDA is used to determine lipid peroxidation levels. The increase in oxidative stress reflected the increase in MDA in renal tissue and preceded the increase in the glomerular and tubular damage score, therefore we then investigated whether one can predict the development of acute renal failure by measurements of BUN, CRE, and uric acid at Day 1 after cisplatin injection. These studies showed a significant correlation between the concentration of MDA and the levels of functional parameters. These findings also suggest that measurement of MDA after cisplatin treatment may be useful for the prediction of cisplatin-induced renal damage.

At the time of chemotherapy, variations in antioxidant activity of natural compounds have an impact on survival and toxicities in different cancers treated with platinum agents [29,30]. In our study, the MDA level was lower in the 50 mg/kg and 100 mg/kg OLE groups compared to the cisplatin group. A significant inhibition of lipid peroxidation in renal cells in the presence of 200 mg/kg was observed ($p < 0.05$). This conclusion fits well with the results published recently by Barbaro et al [31] who suggested that OLE, the main polyphenol in olive oil, contributes to the beneficial health effects. Polyphenols are an important class of bioactive compounds responsible for specific medicinal values. The ingested flavonoids are extensively degraded to various phenolic acids, some of which still

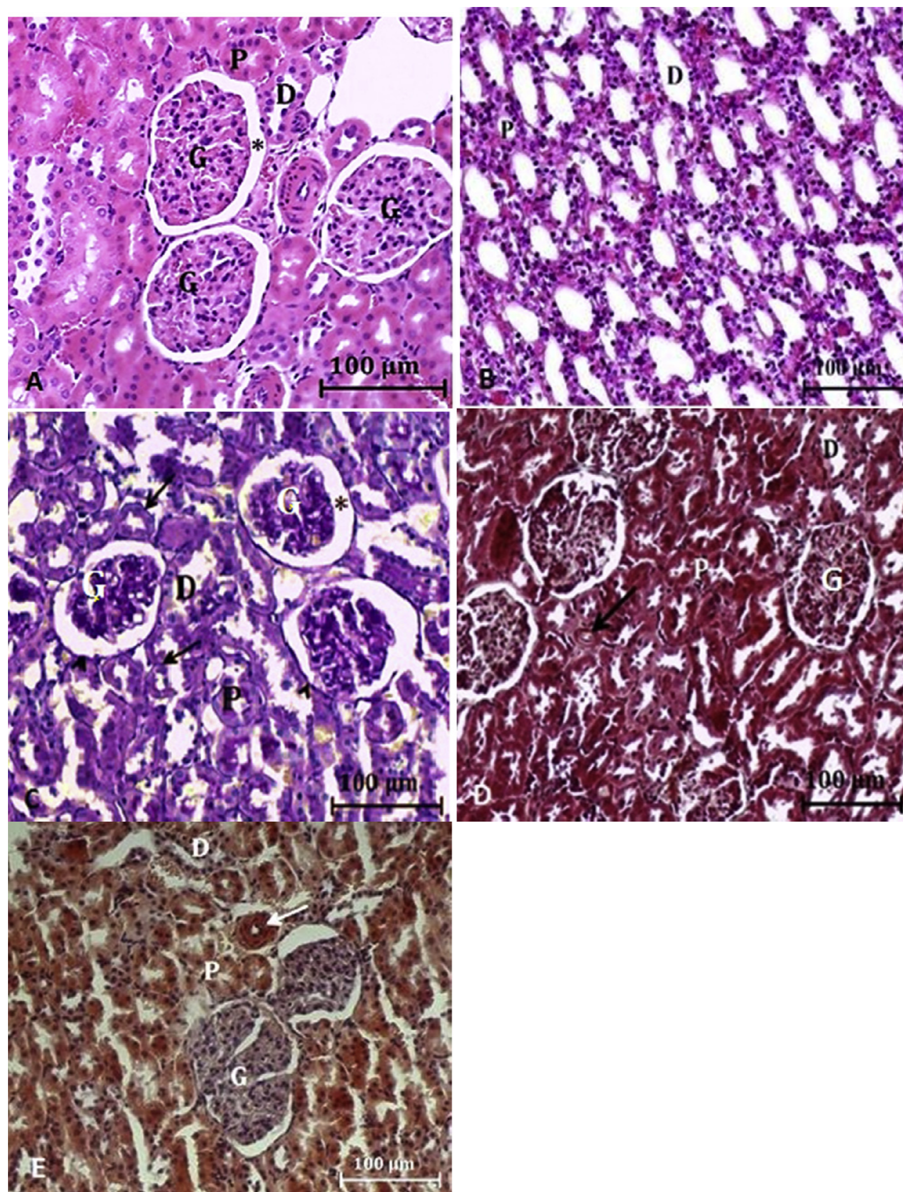


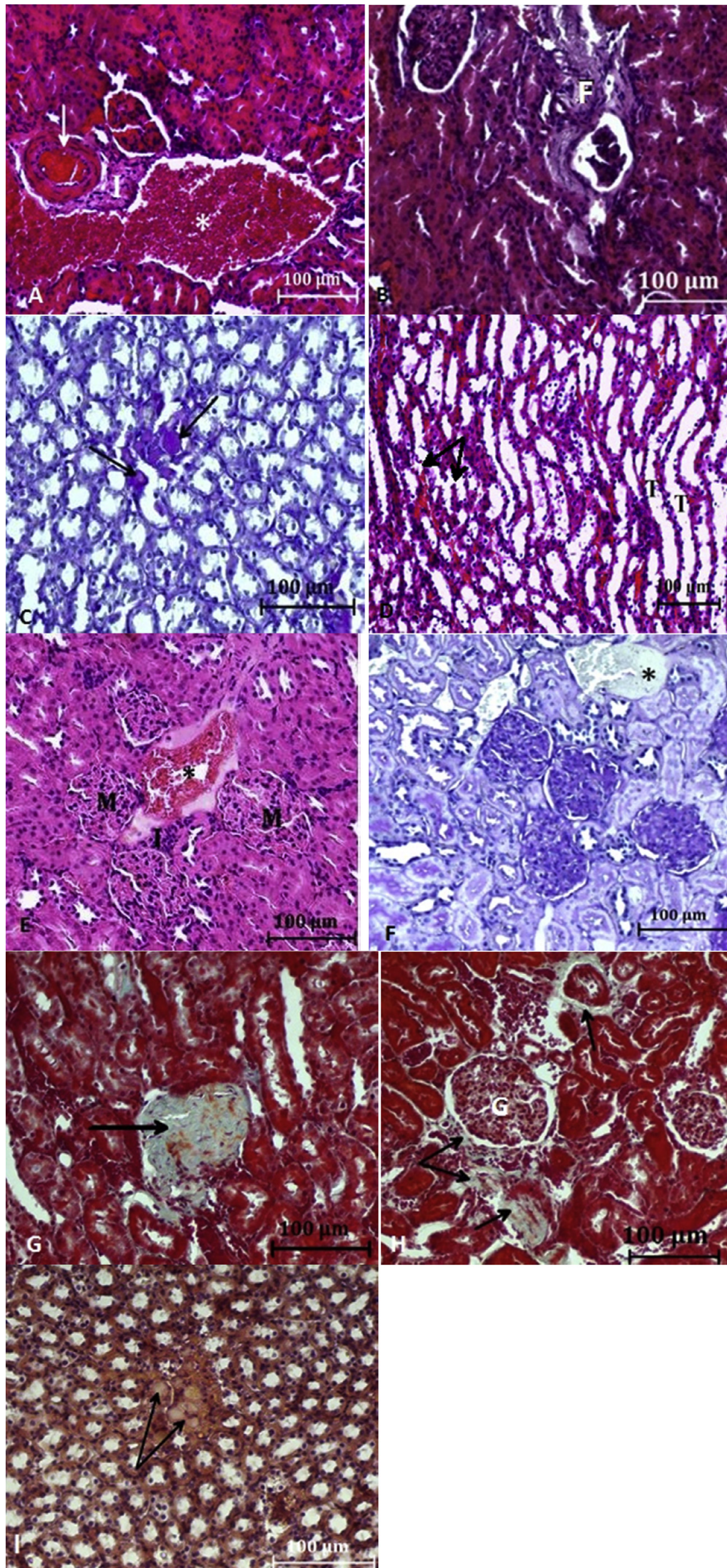
Figure 7 – The control groups with normal kidney histology; (A) Kortex: G = glomerulus, P = proximal tubule, D = distal tubule, * = Bowman space; (B) medulla: D = distal tubule, H&E, ×200; (C) G = glomerulus, P = proximal tubule, D = distal tubule, * = Bowman space, arrows = Tubular basal membrane, PAS, ×200; (D) arrow = arteriole, Masson trichrome, ×200; (E) amyloid, ×200. H&E = hematoxylin-eosin; PAS = periodic acid Schiff.

possess a radical-scavenging ability. Both the absorbed flavonoids and their metabolites exhibit antioxidant activity *in vivo* [32]. The results of the current study showed that treatment of kidney associated with OLE exposure can improve the antioxidant status and balance the oxidative conditions of chemotherapy patients. Although the variations of oxidative factors among rats receiving chemotherapeutic drugs were not noticeable, the OLE therapy considerably increased the levels of TAS and decreased the level of TOS and amended the levels of these factors among cisplatin-treatment rats, similar to those among healthy rats. The significant increase of TAS in the tissues may be dependent on the scavenging of the overproduction of reactive superoxide ion under the oxidative stress induced by cisplatin. Reduced levels of antioxidants and increased oxidative stress in the body are associated with increased risk of cancer [33]. Enzymatic and nonenzymatic antioxidant defense systems play an important role in cellular defense against numerous harmful chemicals produced both exogenously and endogenously [34]. The antioxidants affecting ROS levels are likely to impact patient prognosis after treatment [35,36]. They protect cells from oxidative damage by catalyzing conjugation of ROS [37] and also detoxify various chemotherapeutic agents such as alkylating agents and platinum compounds.

The pharmacological mechanism of cisplatin is associated with its ability to coordinate to genomic DNA, and in particular, guanine residues. Despite its success, cisplatin exhibits several drawbacks including increasing resistance and severe

side effects, which are often associated with the variable repair mechanisms of nuclear DNA and off-target effects on the cytoplasmic components [38–40]. The previously mentioned free oxygen radicals can cause serious damage at the cellular DNA level. The biomarker, 8-OHdG, is a product of DNA damage and is recognized to be the most important marker indicating DNA damage [41,42]. Hydroxyl radicals remove hydrogen from nucleic acids or react with double bonds, resulting in 8-OHdG [43]. Altuner et al [44] showed that DNA damage was increased following single-dose cisplatin (5 mg/kg/d) in rat ovaries. For the first time, we demonstrated that 7 mg/kg/d cisplatin administration induced significant DNA damage to renal cells. According to our results, 8-OHdG score is also a good predictor of tissue damage in cisplatin-induced nephrotoxicity in experimental rats. Previous studies have determined that DNA damage was decreased by antioxidant supplementation [45], and the results of the present study are concordant with these data. OLE exerted its antioxidant effects by reducing ROS-mediated 8-OHdG levels which indicates the prevention of the oxidative DNA damages. This finding was the most important restorative effect of OLE administration.

Cisplatin-induced nephropathy is mainly characterized by tubulointerstitial lesions [46]. There is no scoring system for the report of intensity of cisplatin-induced glomerular damage. In our report, the pathological damages in cisplatin treated rats were in tubules (congestion, tubular necrosis, tubular dilatation, amyloid cast, lymphocyte infiltration, and interstitial fibrosis), and also glomerulus (mesangial matrix expansion and glomerular fibrosis). In line with this, our *in vivo* data showed that glomerular cells are sensitive to cisplatin as tubular cells. Recent studies have indicated that ROS, lipid peroxidation, and depletion of antioxidants have an important role in the pathogenesis of acute lesions induced by cisplatin, and are responsible for various types of oxidative damage in kidney cells [47]. We investigated the effects of TAS and TOS on the kidney in relation to our pathological study. According to our findings, the measurements of TAS in biological tissues will allow us to understand if the protective effect displayed by antioxidants reflects an improvement in endogenous antioxidant defenses and a reduction of chemotherapy risk. We showed that OLE intake is associated with a lower risk of renal damage occurrence with an improved effectiveness in rats exposed to high free radical production. As a matter of fact, lower TAS level might reflect an imbalance of the antioxidant system in the kidney tissue of the cisplatin-induced rat. The fall in TAS is manifested by ROS accumulation in tubular cells, which potentially drives the cell toward apoptosis [48]. As a consequence of chemotherapy intoxication, conductive changes in the cell membrane properties take place. It means that the cell membrane is not in a position to act as a selective barrier, so impairment of ion permeability, enzyme activity, and receptor responsiveness occur [49]. In this regard, it is reported that most of the above-mentioned dysfunctions of cell membrane caused by apoptosis and cisplatin intake are directly or indirectly linked with oxidative stress [50]. Rana et al [51] showed that treatment with antioxidants could increase the eradication of damage caused by cisplatin. It has also been shown that cisplatin-induced renal damage is associated with oxidative stress-facilitated fibrosis [52]. Renal interstitial fibrosis is a major complication of cisplatin treatment, due to the increased accumulation of extracellular matrix proteins [53]. In our study, renal pathological changes were assessed in a detailed manner using H&E, PAS, amyloid, and Masson trichrome staining methods. It is reported that Masson trichrome staining is used to evaluate and quantify the extent of fibrosis in lesions [54]. Accordingly, rat kidneys in the present study were obtained for evaluation of renal fibrosis using this staining method. Our results showed cisplatin-induced renal fibrosis. This effect of cisplatin has been also demonstrated by the other studies with the different dosages of cisplatin applications which have performed acute experiments lasting for 5 days or 7 days with a single ip injection (6 mg/kg or 7 mg/kg) of cisplatin [55–58] and chronic experiments which involved weekly injections (2 mg/kg) for 7 weeks [57,59]. On the other hand, in the present study, cisplatin was administered as a single ip injection of 7 mg/kg in rats on Day 3. In both sets of experiments, cisplatin treatment produced pronounced tubulointerstitial injury, increased infiltration of ED1-positive cells and increased expression of monocyte chemotactic protein-1, α-smooth muscle actin, TGF-β1, phosphorylated Smad3, fibronectin, and gradually developing fibrosis in rat kidney [57]. Similary, another study showed cisplatin administered as a single intravenous dose of 5 mg/kg in female rats for 7 days. Renal fibrosis was assessed by measuring the hydroxyproline content. Significant increases in renal hydroxyproline content were observed in animals receiving treatment with cisplatin [55]. In previous studies, microscopic examinations of kidney tissue were performed only with H&E and PAS-stained tissue sections and the toxic effects of cisplatin were shown in the form of significant tubular damage. According to these reports, cisplatin promoted growth factors such as TGF-β, which plays a key role in the development of morphological changes such as tubulointerstitial fibrosis [56,58]. Moreover, Sherif [58] showed for the first time a significant reduction in renal TGF-β expression following treatment with arjunolic acid, which has an antifibrotic action, at Day 5. The renoprotective effect of alpha-mangostin on cisplatin-induced nephrotoxicity was associated with the attenuation in oxidative/nitrosative stress and inflammatory and fibrotic markers and preservation of catalase activity at Day 7 [56]. Our study revealed that treatment of animals with kidney injury using OLE inhibited the formation of kidney fibrosis as apoptosis at Day 3. The therapeutic effects of OLE were most obvious. In other words, OLE was herein successfully applied to treat the kidney against cisplatin chemotherapy. Additionally, it was reported that cisplatin induces injury to the renal vasculature, leading to the ischemic tubular cell death. Meanwhile, robust inflammatory and oxidative stress responses are activated, further exacerbating nephrocellular damage [60,61]. These results are in agreement with the findings of the present study. Recently, the anti-inflammatory effects of phenolic compounds have been carefully assessed due to their dose-dependent ability [62,63]. Our study showed that OLE decreased cisplatin-induced lymphocyte infiltration in the kidney at lower concentrations; furthermore, in the presence of OLE the inflammation reaction exhibited a concentration-dependent inhibition of the lymphocyte burst. From the pathological score we can see that 100 mg/kg and 200 mg/kg OLE were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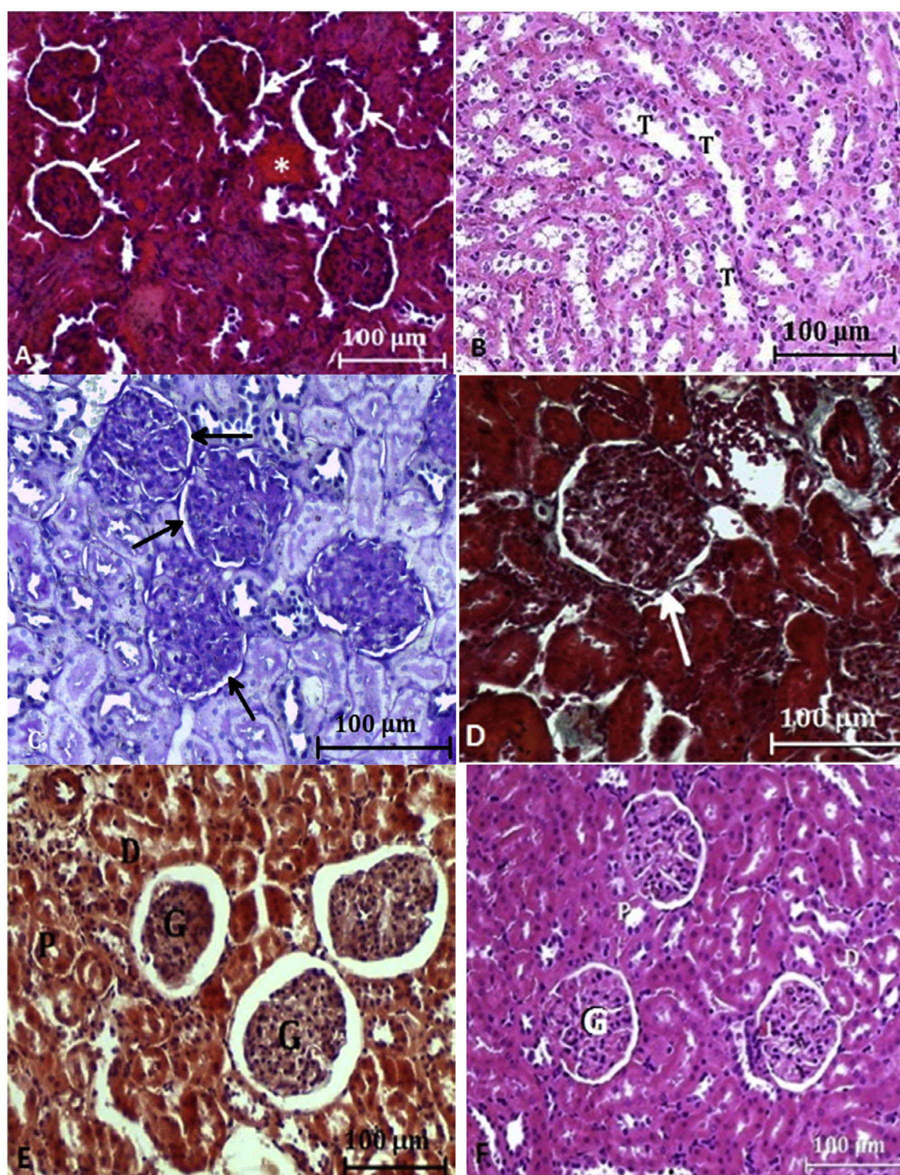

Figure 9 – The kidney histology in CIS + OLE group rats; (A) cisplatin + 50 mg/kg OLE group, arrows = Bowman space, * = congestion; (B) cisplatin + 100 mg/kg OLE group, T = tubules in medulla, H&E, ×200; (C) cisplatin + 100 mg/kg OLE group, arrows = Bowman space, PAS, ×200; (D) cisplatin + 100 mg/kg OLE group, arrows = periglomerular fibrosis, Masson trichrome, ×200; (E) cisplatin + 200 mg/kg OLE group, G = glomerulus, P = proximal tubule, D = distal tubule, H&E, ×200; (F) cisplatin + 200 mg/kg OLE group, G = glomerulus, P = proximal tubule, D = distal tubule, amyloid, ×200. CIS = cisplatin; H&E = hematoxylin-eosin; OLE = oleuropein; PAS = periodic acid Schiff.

much more effective against cisplatin nephropathy than 50 mg/kg OLE. This phytochemical compound shows a potent superoxide anion radical scavenging property and is considered to be an inhibitor of neutrophils respiratory burst [64,65]. In most cases, the toxic effects of amyloid aggregates to exposed cells involve shared early biochemical modifications, including nonspecific membrane permeabilization resulting in intracellular free Ca^{2+} increase, oxidative stress, mitochondria impairment, and eventually apoptosis [66,67]. In conclusion, the renal function deteriorates with vascular

Figure 8 – The kidney histology in CIS group rats; (A) arrow = arteriosclerosis, * = congestion, I = lymphocyte infiltration; (B) F = fibrosis; (C) arrows = amyloid deposits; (D) T = tubular dilatation, arrows = tubular necrosis; (E) M = mesangial proliferation in glomeruli, * = congestion, I = lymphocyte infiltration, H&E, ×200; (F) pale and slightly stained cells, * = amyloid deposit, PAS, ×200; (G) arrow = interstitial fibrosis; (H) arrows = periglomerular fibrosis, G = glomerulus, Masson trichrome, ×200; (I) arrows = amyloid deposits, amyloid, ×200. CIS = cisplatin; H&E = hematoxylin-eosin; PAS = periodic acid Schiff.

Table 2 – Histopathologic scores in kidney of CIS and OLE groups.

Groups	Congestion	Inflammation	Tubular necrosis	Tubular dilatation	Interstitial fibrosis	Mesangial matrix expansion	Glomerular fibrosis	Amyloid
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CIS	4.51 ± 0.54[a]	3.85 ± 0.64[a]	3.54 ± 0.93[a]	3.89 ± 0.67[a]	1.47 ± 0.30[a]	1.29 ± 0.54[a]	3.05 ± 0.78[a]	3.84 ± 1.86[a]
CIS + OLE 50	2.99 ± 0.38[a,b]	3.72 ± 0.49[a]	3.45 ± 0.75[a]	3.74 ± 0.56[a]	1.15 ± 0.25[a]	0.70 ± 0.32[a,b]	2.60 ± 0.55[a]	3.15 ± 0.31[a]
CIS + OLE 100	2.87 ± 0.44[a,b]	2.01 ± 0.28	0.80 ± 0.46[a,b]	0.70 ± 0.43[a,b]	0.90 ± 0.15[a,b]	0.45 ± 0.15[a,b]	1.45 ± 0.62[a,b]	1.34 ± 0.87[a,b]
CIS + OLE 200	0.43 ± 0.21[b]	0.63 ± 0.11[b]	0.59 ± 0.24[b]	0.95 ± 0.27[b]	0.40 ± 0.11[b]	0.24 ± 0.16[b]	0.45 ± 0.37[b]	0.42 ± 0.18[b]

Data are presented as mean ± SD ($n = 7$).
The groups in the same column with different superscript letters are statistically significant ($p < 0.05$) by Tukey's multiple range tests.
CIS = cisplatin; OLE = oleuropein; SD = standard deviation.

amyloid depositions. For these reasons, there are currently many efforts to find naturally occurring molecules, including polyphenols, or to design synthetic ones, which can protect cells against oxidative stress or inhibit amyloid aggregation, mainly at its earliest stages [68]. Our observations revealed that OLE protects cells against the cytotoxic insult caused by cisplatin by virtue of its antioxidant properties. We therefore believe that OLE has an ability to modify the path of amyloid aggregation, to hinder aggregate toxicity, and possible beneficial treatment against cisplatin, which further confirms the multiple benefits potentially coming from extra virgin olive oil consumption and paves the way to further studies on the possible pharmacological use of OLE to prevent or to slow down the progression of kidney damages.

Excessive production of free radicals, such as hydroxyl radicals, superoxide anion, nitric oxide, and hydrogen peroxide and nitrosative stress are also associated with cisplatin-induced renal dysfunction [69]. In the present study, cisplatin treatment caused marked elevation in the levels of the renal markers indicating renal failure. In order to access the renal function in the present study, serum concentration of UA, CRE, and BUN were measured. BUN measures the amount of urea nitrogen, a waste product of protein metabolism in the blood. CRE, a breakdown product of creatine phosphate, is freely filtered by the kidneys and is a measure of glomerular filtration rate [70]. It seems that high UA can be considered as a predisposing factor for metabolic syndrome; thus, it is recommended to measure serum UA in routine tests [71]. If the kidneys do not function properly the serum CRE, BUN, and UA levels increase. Hence, an agent capable of preventing inflammation, oxidative stress, and apoptosis would be of significant clinical interest in the defense against cisplatin-induced nephropathy. The OLE significantly reduced the serum CRE, BUN, and UA levels, suggesting its ameliorating role against cisplatin-induced renal dysfunction. OLE treatment attenuated the raised levels of TOS quite effectively in the cisplatin-treated rat. These observations indicated that OLE inhibited the free radical production in renal tissues by scavenging ROS. A mechanism has explained that OLE could probably act as a chain-breaking antioxidant, terminating the chain reaction of lipid peroxidation [72]. Another study has shown that OLE has two hydroxyl functions and belongs to the ortho-diphenol group. In this case, it can stimulate the nonenzymatic oxidation of superoxide anion into molecular oxygen and consequently dismutase the superoxide radicals [73]. The experimental results of the recent investigations with theoretical achievements show that C3 is a unique antioxidant active site in the OLE molecular structure [74,75]. This striking character of C3 is arisen from its special geometry, resonance, and the radical structural stability, which is attributed to the presence of sugar and catechol moieties, and the intramolecular hydrogen bond in between. This position has a significant role in antioxidant activity for scavenging the small and nonionic radicals [76,77]. In addition to their antioxidant activity, the potent diuretic activity of vegetable oil fractions has been indicated by Somova and colleauques [78] and this activity is attributed to Na^+ and K^+ content in distal tubules. Therefore, the restroration effect of OLE in cisplatin induced kidney failure may be associated with its potent diuretic activity since it has been known that cisplatin administration caused hyponatremia.

In conclusion, the findings of the present study indicate that OLE could be a novel and effective approach for preventing cisplatin nephrotoxicity due to its antigenotoxic, antioxidative, and anti-inflammatory actions not only in tubular cells but also in glomerular cells. On the basis of long-term experience and extensive clinical data, OLE may be a candidate for the management of cisplatin-induced DNA damage, pathological lesions, and functional disorders in the kidney.

Conflicts of interest

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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