Regular Article

Opuntia ficus indica Fruits Ameliorate Cisplatin-Induced Nephrotoxicity in Mice

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This study aims to determine the potential renal protective effects of *Opuntia ficus-indica* (L.) Miller (OFI) fruits against cisplatin-induced nephrotoxicity in mice. The antioxidant activity of OFI methanol extract was calculated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) free radical scavenging assays. Furthermore, the LC-mass spectroscopy (MS) analysis of the OFI fruit extract was carried out. Mice were treated with OFI extract (250 mg/kg) for 10 d and injected with a single dose of cisplatin (20 mg/kg) on the 7th day. The blood samples were collected to measure blood urea nitrogen (BUN) and serum creatinine level on the 10th day. Their kidneys were removed for histopathological examination. The renal morphological alterations were assessed through the mesangial matrix index and transmission electron microscopy (TEM). The OFI fruit extract showed significant *in vitro* antioxidant activity. In further, it was revealed that the cisplatin-induced nephrotoxicity in mice was ameliorated; this outcome was supported by both histological examination results and the depicted reduced levels of BUN and serum creatinine. The potent antioxidant compounds which were detected in the extract of OFI fruits such as myricetin, quercetin, luteolin might be responsible for the observed renoprotective effect. The results clarified that the OFI fruit extract could ameliorate cisplatin-induced renal toxicity in mice *via* including antioxidant and renoprotective compounds.

Key words cisplatin; nephrotoxicity; opuntia; methanol extract; mouse

INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum II) is an effective platinum anti-cancer drug generally used in the treatment of testicular, ovarian, urinary, and bladder cancers and solid tumors.[1,2] As an alkylating agent, cisplatin causes cell death to cancer cells after crosslinking to DNA. Although, cisplatin is widely applied as anti-cancer therapy, its use is limited by its unwanted side effects such as neurotoxicity, ototoxicity, nausea and vomiting, and nephrotoxicity.[3] On cisplatin use, the most important dose-limiting factor is the possible acute or chronic kidney damage. Other factors are the decreased glomerular filtration rate, oliguria, transient proteinuria, increased serum blood urea nitrogen (BUN), and creatinine levels. Renal function disorder occurs in almost 25–30% of cases after reaching a dosage of 80 mg/cm.[2,4] Nephrotoxicity induced by cisplatin is associated with high morbidity and mortality.[5] Renal functions are especially affected by cisplatin as a result of the high amount of cisplatin concentration in the kidneys.[1] The recommended strategy for the prevention of renal dysfunction in cisplatin-based chemotherapy involves the maintenance of the urine flow by hydration with saline before and after cisplatin chemotherapy.

The mechanisms responsible in cisplatin-induced nephrotoxicity have been reported as multifactorial, including inflammation, oxidative stress, and apoptosis. Inflammatory mechanisms and mediators such as tumor necrosis factor-2 alpha (TNF-α) and interleukin-1 beta could play an important role in the pathogenesis of cisplatin nephrotoxicity.[5,6] Production of reactive oxygen species by cisplatin in the kidney is crucial to the progression of nephrotoxicity.[1] The proposed mechanism of cisplatin-induced nephrotoxicity includes activation of p53 tumor suppression protein. It has been shown that oxidative stress could link to cisplatin-induced p53 activation.[7]

Various scientific papers represent the protective activity of common antioxidants such as *N*-acetylcysteine, vitamin E, and carvedilol,[1] plant products, and inhibitor of signaling pathways in cisplatin-induced renal damage.[8] Therefore, the effort for testing phytochemicals, as possible agents which can decrease the undesirable side effects of antineoplastic agents, should be reinforced.

The prickly pear cactus (*Opuntia ficus-indica* (L.) Miller) which belongs to the Cactaceae family, is known as "kaynanadili, firenkinciri, hintinciri" in Turkey.[9] The cactus pear *Opuntia ficus-indica* (OFI), originated from the American continent, was introduced into the Mediterranean and Aegean Region countries during the 16th century.[10] In general, the prickly pears are consumed as fresh fruits, juices or jams. In furher, the prickly pear cactus is an important nutrient and food source because of its natural antioxidants which can be protective against oxidative damage.[11] Phytochemical

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compounds found on Prickly pear fruits and cladodes are vitamins, carotenoids, betalains, and polyphenolic compounds. These molecules show various biological activities such as anti-cancer, anti-diabetic, anti-inflammatory as well as neuroprotective effects.[12,13] Consequently, OFI has gained importance in recent years due to these properties.[14]

The renal toxicity is the major barrier in the clinical cisplatin treatment. Thus, identifying how to control renal toxicity while maintaining the antitumor activity is the primary spotlight in clinical investigations. In this study, the total phenolic compounds and flavonoid contents of OFI fruits were determined and investigated in respect to their antioxidant and nephroprotective activities on cisplatin-induced renal toxicity in mice.

RESULTS AND DISCUSSION

Total Phenolic and Flavonoid Content In this study, the phenolic compounds of cactus pear fruit extracts were measured by Folin–Ciocalteu reagent. The total phenolic value, which was obtained from the OFI fruit methanol extract, found to be 363.20 ± 0.02 mg gae/100 g. It was revealed that the total flavonoid content of the OFI fruit extract was measured at 1490.34 ± 0.05 mg qe/100 g from fruit extract.

***In Vitro* Antioxidant Assays** The free radical scavenging ability of cactus pear fruits was determined by measuring the disappearance of colored synthetic radicals as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS). The obtained results of both free radical scavenging assays of OFI fruit extract were presented in Table 1. In the test solution, OFI fruit extract reduced the stable free radical ABTS and DPPH presented by IC_{50} value and defined as the concentration of the antioxidant required to scavenge 50% of ABTS and DPPH. It was found that OFI fruits are endowed with significant activity, as low IC_{50} value was obtained by the ABTS and DPPH colorimetric assay. These results showed that the OFI methanol extract has a significant free radical scavenging activity compared to the standard compound-ascorbic acid which is a potent antioxidant.

LC-Mass Spectroscopy (MS) Analysis The phytochemical constituents of the cactus pear fruits were analyzed using LC-MS which led to the identification of different compounds. The LC-MS result is checked with the standard LC-MS chromatogram and the obtained standard chromatogram is given at Fig. 1.

Table 2 shows the phenolic compounds found on OFI fruit methanol extract. For data analysis, the positive identification of each compound was based on retention time and mass information of library score standards. It can be reported that the extract contains 3 different compounds according to these standards. The analysis revealed myricetin (**5**), quercetin (**6**), and luteolin (**8**) as the main flavonoid components. Furthermore, Fig. 2 depicts the LC-MS chromatogram obtained of OFI-fruit methanol extract. Herein, myricetin (retention time (R.T.): 11.44) (**5**), quercetin (R.T.): 15.28) (**6**), and luteolin (R.T.: 19.16) (**8**) were detected in the cactus pear fruits according to LC-MS results (Fig. 2). These compounds were determined by comparing them with the known standards (Fig. 1). Other standards (Gallic acid (**1**), Luteolin-7-*O*-glycoside (**2**), Coumaric Acid (**3**), Rosmarinic Acid (**4**), Apigenine (**7**)) could

Table 1. ABTS and DPPH Scavenging Activities of OFI Fruit Extract

	OFI Fruit extract	References
	$IC_{50} \pm$ S.D. (mg/mL)	
ABTS	2.46 ± 0.04	3.84 ± 0.04 (Trolox)
DPPH	4.12 ± 0.03	4.67 ± 0.04 (Ascorbic acid)

Table 2. The Identified Phenolic Compounds of OFI Fruit Methanol Extract According to Their Retention Time (R.T.), Area and Base Peak

Compound	R.T.	Area	Base peak (*m/z*)
Myricetin	11.436	6268	316.93
Quercetin	15.250	6926446	301.02
Luteolin	19.153	39288	285.03

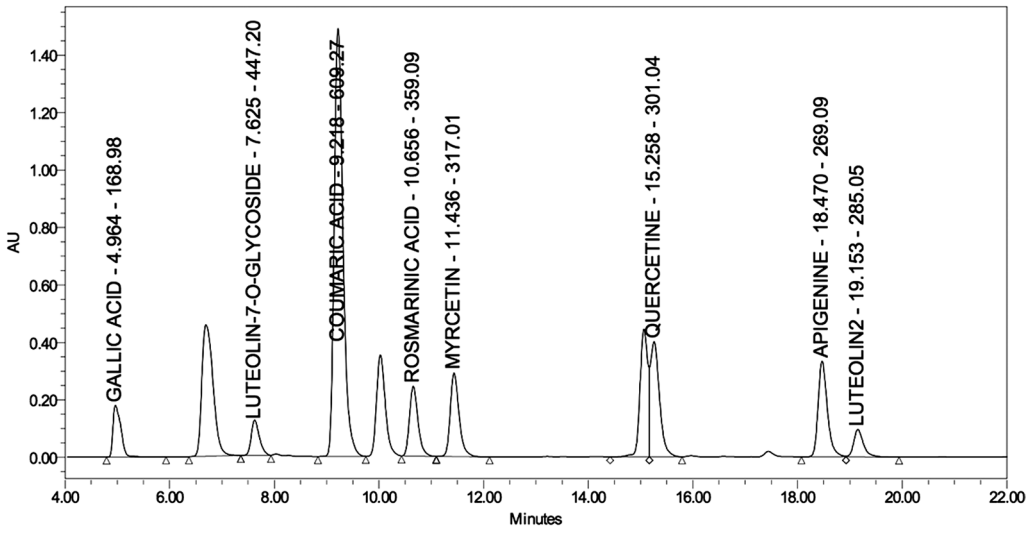

Fig. 1. LC-MS Standard Chromatogram

Standards: Gallic acid (R.T.: 4.96) (**1**) ; Luteolin-7-*O*-glycoside (R.T.: 7.62) (**2**); Coumaric acid (R.T.: 9.21) (**3**); Rosmarinic acid (R.T.: 10.65) (**4**); Myricetin (R.T.: 11.43) (**5**); Quercetin (R.T.: 15.25) (**6**); Apigenine (R.T.: 18.47) (**7**); Luteolin (R.T. 19.15) (**8**) (R.T. = Retention time).

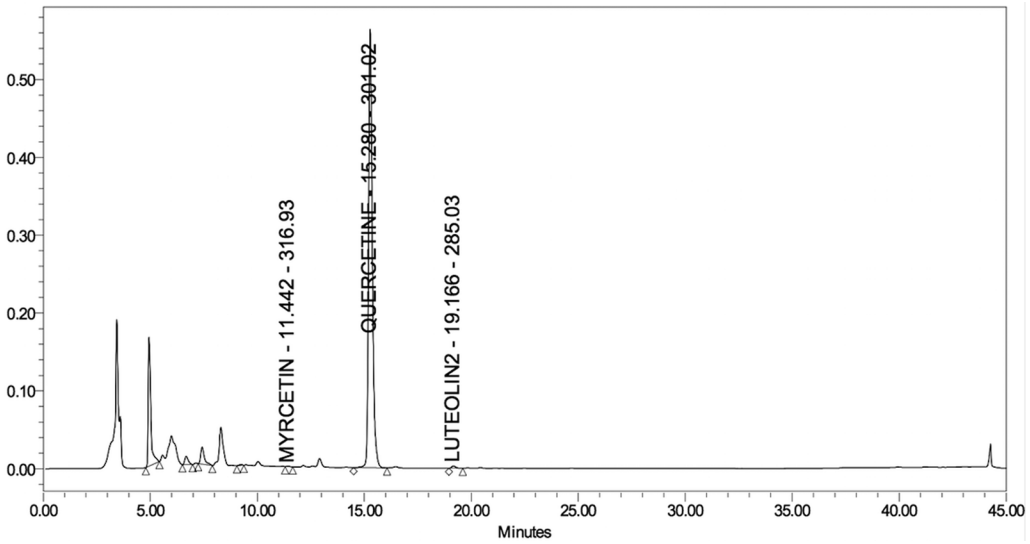

Fig. 2. OFI-Fruit Methanol Extract LC-MS Chromatogram

Peak **1** (R.T.: 3.15)-NI; Peak **2** (R.T.: 4.94)-NI; Peak **3** (R.T.:6.68)-NI; Peak **4** (R.T.: 7.42)-NI; Myricetin (R.T.: 11.44) (**5**); Quercetin (R.T. : 15.28) (**6**); Luteolin (R.T. : 19.16) (**8**) (R.T. = Retention time). *NI: Not Identified.

not be determined in the cactus pear fruits extract. Nonetheless, some organic compounds which have not been identified can be also found (Peaks **1**–**4**). Hence, the antioxidant capacity of the extract could be linked to both identified flavonoid and un-identified compounds. In previous studies, it was reported that OFI had antioxidant potential due to phenolic compounds.[11,15,16] On the other hand, the synergetic effect of all the depicted compounds is possibly involved in the antioxidant properties of the extract.

In a previous study from our lab, OFI fruit *n*-hexane extract was analyzed using GC-MS/GC-flame ionization detector (FID) to determine the volatile constituents of the extract. The *n*-hexane extract was found to be rich in borneol which is associated with the observed antioxidant activity.[17,18]

Effect of OFI on BUN and Serum Creatinine Levels The serum creatinine and BUN levels are two main indicators for the monitoring of the renal hemostasis.[19] The effect of cactus pear fruits on cisplatin-induced renal damage was investigated by measuring the levels of BUN and creatinine at 72 h after a single dose of cisplatin administration. The results of blood samples analysis stated that cisplatin (Cis) group displayed a significant increase in serum creatinine (1.47 ± 0.47) ($p < 0.001$) and BUN (236.6 ± 33.01) ($p < 0.001$) levels as compared to control (Con) group. It can be said that the induction of severe nephrotoxicity was indicated by elevated levels of BUN and serum creatinine in the cisplatin (Cis) group. However, both OFI pre-treatment (OFI-Cis and OFI-Con) groups did not show any significant difference compared to the control group. On the other hand, the pre-treatment with OFI fruits exhibited a significant decrease on BUN (84.73 ± 19.8) ($p < 0.001$) and serum creatinine (0.43 ± 0.14) ($p < 0.001$) levels on the cisplatin treated (OFI-Cis) mice compared with the separate cisplatin treated (Cis) mice group (Figs. 3, 4).

Histological Results

Light Microscopy

The light microscopy indicated that control (Con) and OFI fruit pre-treated (OFI-Con) groups showed no abnormal findings. Moreover, the renal glomeruli and tubules of cisplatin (Cis) group depicted degenerative differences whereas the uri-

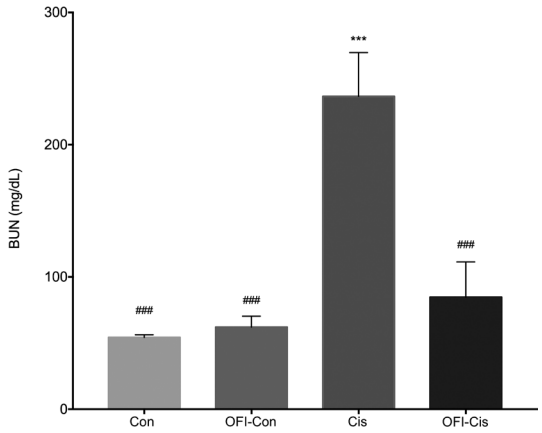

Fig. 3. Effect of OFI Pretreatment on BUN Levels in Control (Con), Cisplatin (Cis), OFI Fruit (OFI-Con), OFI Fruit-Cisplatin (OFI-Cis) Groups

Each data point represents the mean ± standard error of the mean (S.E.M.). ($n = 10$), $^{***}p < 0.001$ *vs.* control group, $^{###}p < 0.001$ *vs.* cisplatin group.

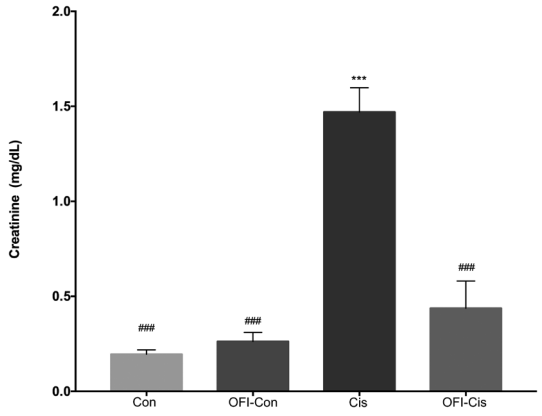

Fig. 4. Effect of OFI Pretreatment on Creatinine Levels in Control (Con), Cisplatin (Cis), OFI Fruit (OFI-Con), OFI Fruit-Cisplatin (OFI-Cis) Groups

Each data point represents the mean ± S.E.M. ($n = 10$), $^{***}p < 0.001$ *vs.* control group, $^{###}p < 0.001$ *vs.* cisplatin group.

nary spaces were reduced. Nevertheless, the pre-treatment with OFI fruit extract on cisplatin-applied mice (OFI-Cis) showed a protective effect since normal tubules and glomeruli were depicted. The mesangial matrix expansion levels of the healthy and renal-damaged mice with/without OFI fruit pre-treatment are displayed in Fig. 5.

According to the periodic acid Schiff (PAS) staining results, even though the OFI pre-treatment on cisplatin-applied mice (OFI-Cis) ($p < 0.01$) presented higher mesangial matrix index compared to the control group, the cisplatin-treated group (Cis) ($p < 0.001$) exhibited the highest mesangial matrix index among all groups. The cisplatin-applied OFI treated (OFI-Cis) mice group showed a significant increase ($p < 0.05$) in the mesangial matrix fraction, compared to the Cisplatin-applied untreated (Cis) mice (Fig. 6).

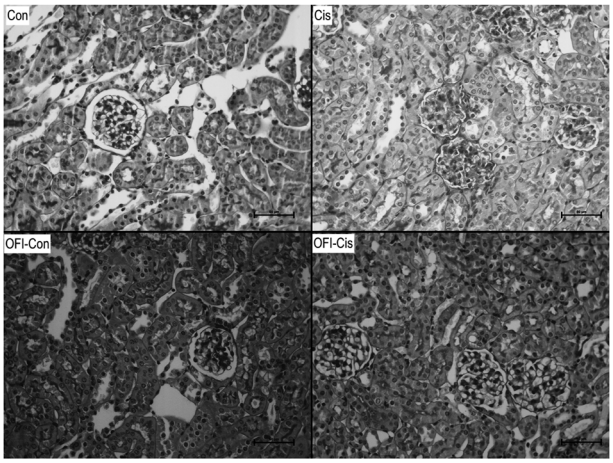

Fig. 5. Representative Images of Kidney Tissues of Control (Con), Cisplatin (Cis), OFI Fruit (OFI-Con), OFI Fruit-Cisplatin (OFI-Cis) Groups

Transmission Electron Microscopy The glomeruli in the

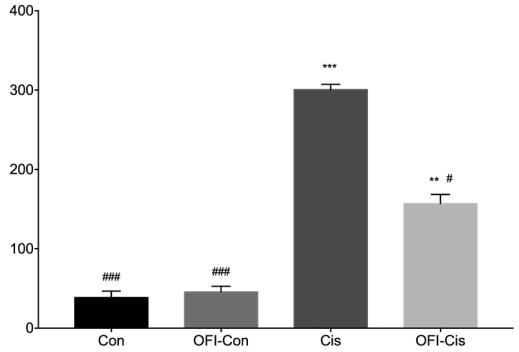

Fig. 6. The Effects of OFI Treatment on Mesangial Matrix Expansion in Nephrotoxic Mice

All data were expressed as mean ± S.E.M. ($n = 10$), $^{***}p < 0.001$, $^{**}p < 0.01$ *vs.* control group, $^{\#\#\#}p < 0.001$, $^{\#}p < 0.05$ *vs.* cisplatin group.

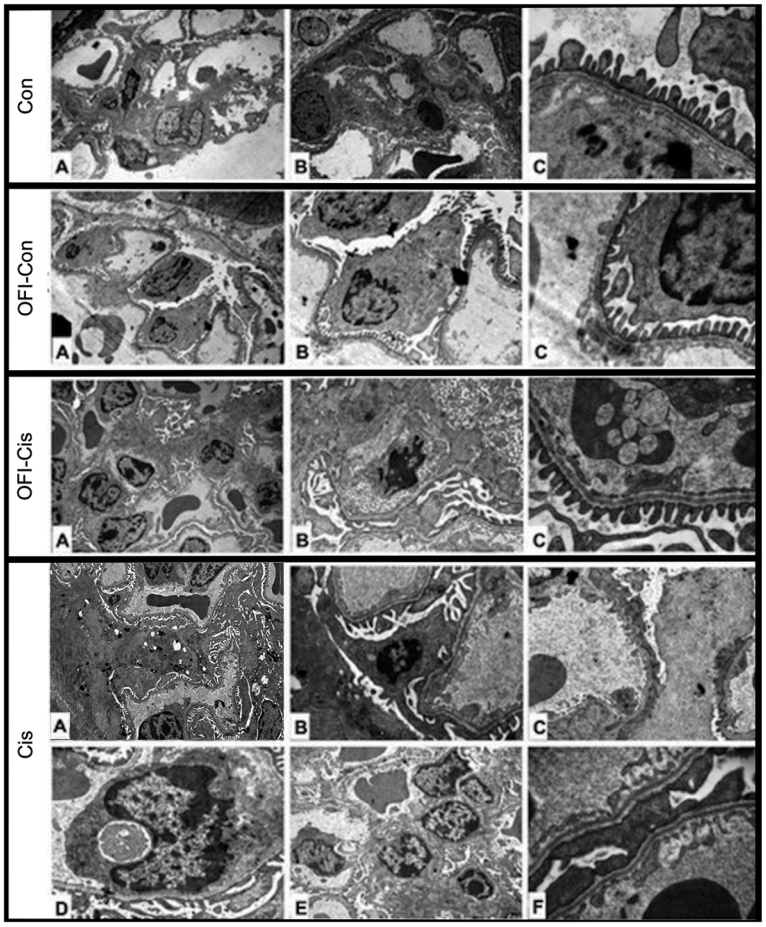

Fig. 7. Electron Micrographs of Different Glomeruli of Experimental Groups at Various Magnifications

Con; A ×4000; B ×5000; C ×30K, OFI-Con; A ×5000; B ×12K; C ×30K, Cis; A ×5000; B ×20K; C ×15K; D x10K; E ×20K; F ×40K, OFI-Cis; A ×5000; B ×15K; C ×40K.

control (Con) group showed normal structure (Fig. 7: Con-A). Podocytes did not show any degeneration, and pedicels were observed with regular intervals. Glomerular basement membrane (GBM) presents normal thickness. The mesangial matrix (MM) and the mesangial cells (MCs) depicted also normal distribution (Fig. 7: Con-B,C). Podocytes in normal mice with OFI pre-treatment (OFI-Con) group showed normal structure (Fig. 7: OFI-Con-A). In further, apical villous structures were observed in some podocytes (Fig. 7: OFI-Con-B). Pedicels are depicted with regular intervals (Fig. 7: OFI-Con-C). The OFI fruit pre-treatment cisplatin-applied mice (OFI-Cis) were morphologically close to the control group (Fig. 7: OFI-Cis-A). However, apoptotic endothelial cells were found in some places (Fig. 7: OFI-Cis-B). The pedicels exhibited a regular structure (Fig. 7: OFI-Cis-C). The podocytes in glomeruli found as hypertrophic in cisplatin-treated (Cis) group. Moreover, vacuolization, lysosomal structures, and mitochondrial hyperplasia were also observed in these hypertrophic podocytes (Fig. 7: Cis-A). A small number of apoptotic podocytes was found whereas GBM thickening and hump structures were found in the sites near these apoptotic podocytes (Fig. 7: Cis-B). Pedicels formed long strips of fusion and the cytoplasm in these regions showed a more electron-dense image. Invaginations and thickening were seen in GBM in the same areas (Fig. 7: Cis-C, F). Autophagic vacuoles in the cytoplasm of apoptotic endothelial cells were noted (Fig. 7: Cis-D). In addition to the increased mesangial cell proliferation and mesangial matrix, also apoptotic mesangial cells were found (Fig. 7: Cis-E).

DISCUSSION

Opuntia ficus-indica-prickly pear-fruits exhibit several pharmacological effects such as hypoglycemic, anti-ulcer, anti-allergic and cancer chemoprevention activities.[20] Up to date there is no study which evaluated the effect of prickly pear in cisplatin-induced nephrotoxicity. In this investigation, the antioxidant capacity, photochemical screening and *in vivo* renal protective effect of OFI fruits were also studied.

Cisplatin is chiefly excreted *via* renal route whereas its accumulation is much higher in the epithelium of renal tubules than in circulation, inducing severe renal toxicity.[4] The mechanism of cisplatin-induced cellular impairment is a sophisticated process including apoptosis, fibrogenesis, inflammation, and oxidative stress.[3] The free radicals can lead to modifications in cell complex and function which affect tissue and organ function.[4] According to the obtained results, OFI fruits possess an abundant phenolic and flavonoid content. Previous studies also demonstrated that prickly pears are rich in polyphenols, flavonoids as well as ascorbic acid and vitamin E.[21,22] In agreement with earlier studies, a similar level of phenolic and flavonoid compounds has been detected in *O. ficus indica* prickly pear and their juice.[23]

In general, two different analyzing methods are used for evaluating antioxidant activity.[24] In this study, the antioxidant activity was analyzed by ABTS and DPPH free radical scavenging activity tests. Both analyzing methods showed that OFI fruit extract exhibit significant antioxidant activity compared to ascorbic acid. Similarly, significant antioxidant activity was also observed in a previous study conducted with OFI fruit puree.[25] Vitamins, phenolic, and flavonoid components are the main antioxidant compounds of the plants.[26]

It has been reported that the cactus pear fruits contain bioactive compounds such as betaxanthin, indicaxanthin, betacyanin, kaempferol, quercetin and isorhamnetin. Other molecules are dihydroquercetin, eucomic acid, ferulic acid, isorhamnetin, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, isorhamnetin-3-*O*-lyxose-rhamnose-glucoside, isorhamnetin-3-*O*-rhamnose-rutinoside, kaempferol-3-*O*-rutinoside, piscidic acid, rutin, quercetin 3-methyl ether and sorhamnetin triglycoside.[16,21,27–29] These compounds display antioxidant and anti-inflammatory properties and they have been proposed to be responsible for the biological activities of OFI fruits.[16,21,27–29] In this investigation, myricetin (**5**), quercetin (**6**), and luteolin (**8**) were detected to the cactus pear fruits according to LC-MS results (Fig. 2). These compounds were determined in comparison with the known standards (Fig. 1). Other standards have not been determined in the cactus pear fruits extract. Moreover, borneol was also detected in our previous study.[18] The phyto-antioxidants are reported to perform protection without compromising the antineoplastic ability of chemotherapeutic agents.[3]

Myricetin possesses biological activities such as antioxidant and anti-inflammatory effects. Hassan *et al.* demonstrated that myricetin treatment before cisplatin injection showed a remarkable decrease in levels of serum creatinine and serum BUN, indicating improvement of kidney functions in mice.[30] It was previously reported that myricetin was also found in cladode parts of OFI cactus[31] and OFI fruit peels.[32] However, OFI fruits are poorly investigated about myricetin.

Quercetin is a potent oxygen free radical scavenger and a bioflavonoid component of OFI fruits.[33] A previous report demonstrated that co-treatment with quercetin partially prevented all the renal effects of cisplatin, whereas it did not impair its anti-tumor activity in rats.[34] A similar study, also showed amelioration of cisplatin-induced nephrotoxicity and improved therapeutic efficacy of the drug.[35] Thus, we believe that the extract will not reduce CDDP antitumor effect in Balb-c mouse cancer model, if studied.

Luteolin is a well-known phenolic compound, which exhibits numerous pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial activities. It has been reported that luteolin significantly improve the cisplatin-induced nephrotoxicity by suppressing oxidative stress, inñammation, apoptosis[36] and due to down-regulation of the p53-dependent apoptotic pathway in the kidney.[7]

The injection of cisplatin mainly produces proximal tubular necrosis and renal epithelial cell apoptosis.[1] Consequently, cisplatin can cause acute renal injury and extreme hydrogen peroxide in the renal cortex,[4] which induces an increase in serum creatinine and BUN. Therefore, kidney function was observed by measuring the serum creatinine and BUN levels after injection of cisplatin.[37] In the present study, the single dose cisplatin treated kidney damaged mice and non-treated normal mice were used as controls. The mice were gavaged with OFI fruit extract (250 mg/kg) for 10 consecutive days. It can be reported that no toxic effect was observed during OFI fruit pre-treatment. According to the data, the restored renal function was monitored through improved serum BUN and creatinine levels on cisplatin-applied mice with OFI pre-treatment. Cisplatin-treated animals demonstrated a significant rise in serum urea and creatinine levels that they can be associated with the reduced glomerular filtration ratio or due to

secondary elevated levels of the reactive oxygen species which induce mesangial cells contraction.[38]

The obtained results from the histopathological examination revealed that OFI pre-treatment significantly reduced mesangial matrix expansion in cisplatin-treated mice. The histological examination of renal tissue and evaluation of transmission electron microscopy demonstrated that OFI pre-treatment in cisplatin-treated mice almost ameliorate normal tissue organization with cellular integrity. In agreement with the present findings, Alimi *et al.* reported the ameliorative effect of OFI fruits on ethanol-induced oxidative stress in rats, that can result from inhibition of free radicals chain reactions or from the enhancement of the endogenous antioxidant activity.[22] Moreover, Galati *et al.* also demonstrated a similar protective effect which is related to antioxidant ingredients of OFI fruits on carbon tetrachloride-induced liver damage.[39]

Oxidative stress is considered to perform a key role in cisplatin-induced renal toxicity.[40] Many researchers proposed that antioxidants molecules as naringenin, rosiglitazone, quercetin, co-enzyme Q_{10} and riboflavin can eliminate the undesirable side effects of cisplatin.[3] The *O. ficus indica* has shown remarkable therapeutic effects as a result of its potent antioxidant and free radical scavenging activity. Hence, the OFI was screened for the intervention study against nephrotoxicity due to cisplatin. The OFI fruit contains several antioxidant compounds such as ascorbic acid, myricetin, quercetin, luteolin. All these substances can act synergistically because of their antioxidant activity. Consequently, the renal protective effect of *O. ficus indica* fruits might result from the high content of antioxidant compounds.

MATERIALS AND METHODS

Materials Cisplatin was purchased from Koçak Farma, Istanbul, Turkey. The standard chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and the HPLC-grade solvents were purchased from Merck. Methanol was purchased from Sigma-Aldrich, Germany. All other reagents and solvents used were of analytical grade.

Plant Material and Preparation of Extract OFI fruit samples were collected from Turunç, Marmaris, Turkey (Date: 04.08.17). The plant material was identified by Derya Çiçek Polat and the voucher specimen has been deposited at the Herbarium of the Ankara University, Ankara, Turkey (Voucher specimen no: 28753). The pinkish fruits were thinly cut and dried. The samples were powdered and extracted with methanol on a magnetic stirrer (Heidolph MR3001, Germany) (200 g sample, 200 mL×3) followed by filtration with filter paper. The methanol extract was distilled using a rotary evaporator (Heidolph WB2000, Germany). The extract yield of fruit was calculated as 8.98% (w/w).

***In Vitro* Antioxidant Assays**

DPPH Scavenging Assay

To determine the antioxidant ability of OFI fruit extract, the DPPH radicals were utilized according to the spectrophotometric protocol.[41] The absorbance was read at 517 nm using a spectrophotometer. The radical scavenging activity was calculated according to the following equation:

$$\%\mathrm{DPPH}_{\mathrm{inhibition}} = \left[\frac{(\mathrm{Absorbance}_{\mathrm{control}} - \mathrm{Absorbance}_{\mathrm{test\ sample}})}{\mathrm{Absorbance}_{\mathrm{control}}}\right] \times 100$$

All experiments were done in triplicate. Ascorbic acid was served as the positive control. IC_{50} rates were detected from a calibration curve.[26]

ABTS Scavenging Assay

ABTS radical scavenging activity of OFI fruit extract was determined according to Re *et al.*[42] Stock ABTS·solution was composed by reacting aqueous of ABTS (7 mM) with potassium persulfate solution (2.45 mM). The mixed solution was incubated for 12–16h in the dark at room temperature. The absorbance of reaction mixtures was measured at 734 nm. Three independent experiments were performed. An analogue of vitamin E, Trolox was used as the positive control.[24] The results were compared with Trolox and expressed as IC_{50} as follows:

$$\%\mathrm{ABTS}_{\mathrm{inhibition}} = \left[\frac{(\mathrm{Absorbance}_{\mathrm{control}} - \mathrm{Absorbance}_{\mathrm{test\ sample}})}{\mathrm{Absorbance}_{\mathrm{control}}}\right] \times 100$$

Total Phenolic and Flavonoid Contents of the Extract Folin–Ciocalteu method was used to determine total phenolics of the fruit extract. The mixture was prepared with fruit extract (5 mL), Folin–Ciocalteau's reagent (0.25 mL) and Na_2CO_3 (0.2 mL) and allowed to stand for 15 min at 45°C. The absorbance reading of samples was performed at 765 nm. A calibration curve ($R^2 = 0.9811$) was used for the calculation of total phenolic content (TPC) of the extract[26] and the result was expressed as mg gallic acid equivalent (gae)/100 g extract.[43]

The aluminum chloride colorimetric technique was used to determine the total flavonoid content of OFI fruit methanol extract. The absorbance of the mix was read at 510 nm. The flavonoid content was measured by the calibration curve ($R^2 = 0.9978$)[24] and the outcome was displayed as mg quercetin equivalent (qe)/100 g extract. Three independent experiments were performed for each analysis.

LC-MS Analysis The methanol extract was analyzed *via* LC-MS for the identification of its chemical compounds. Firstly, the extract was filtrated through 0.22 μm membrane filters. For the preparation of calibration curves of standards, 5 mg of each standard was dissolved in 5 mL MeOH and filtered. By diluting the stock solutions, five different concentrations of standards were prepared.

The extract was analyzed using LC-MS on a single quadrupole mass spectrometer (1200 LC, Agilent). An Agilent C18 column (4.6 × 250 mm 5 μm) was used and its temperature was maintained at 40°C. The mobile phase was A: Acetonitrile : Water : Formic acid (10 : 89 : 1) B : Acetonitrile : Water : Formic acid (89 : 10 : 1). The gradient elution established in the time frame 0–30 min, B% 15–45 and flow rate 0.7 mL/min. The injection volume is 20 μL.[44]

Laboratory Animals The balb-c mice (25–28g) were procured from Medipol University, MEDITAM, Istanbul, Turkey. They were fed *ad libitum* water and food under laboratory conditions and kept in standard cages (23 ± 1°C and 12 h/12 h dark–light cycle). The animal experiment was performed according to the local ethical committee (No: 27.12.17-76). All experiments involving animals were conducted in accordance

with the ethical guidelines for the care of laboratory animals.

Experimental Groups The fourty mice were equally separated into four groups ($n = 10$) and grouped as follows: Control (Con) group: gavaged with saline (0.5 mL) for 10 d and injected a single dose of saline (0.5 mL, intraperitoneal (i.p.)) on the 7th day. Cisplatin (Cis) group: gavaged with saline (0.5 mL) for 10 d and injected a single dose of cisplatin (20 mg/kg, i.p.) on the 7th day. OFI fruit (OFI-Con) group: gavaged with OFI fruit extract (250 mg/kg) for 10 d and injected a single dose of saline (0.5 mL, i.p.) on the 7th day. OFI fruit-Cisplatin (OFI-Cis) group: gavaged with OFI fruit extract (250 mg/kg) for 10 d and injected a single dose of cisplatin (20 mg/kg, i.p.) on the 7th day.

Determination of BUN and Creatinine The animals were sacrificed on the 10th day, 72 h after cisplatin administration. Their blood samples were collected in test tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Afterwards, the blood samples were centrifuged at 3000 rpm for 10 min. The plasma was separated and stored at −4°C for further analysis. The serum creatinine and BUN levels were measured using a colorimetric assay kit according to the manufacturer's instructions. The BUN and serum creatinine levels were measured as indicators of kidney function.

Histopathology Examination

Light Microscopy

The kidneys were sectioned and fixed in 10% formalin, dehydrated and embedded in paraffin. Tissues were sectioned at 5 μm and stained with PAS. The kidney tissues were evaluated for a semi-quantitative scoring method by a researcher-blinded to treatments of mice, as explained by Ayla *et al.*[45] The photographs of sections were taken at different magnifications in a Nikon Eclipse Ni research microscope, Amsterdam, Netherlands, fitted with Nikon DS-Fi2 model digital camera and used Nikon Sight D5 V3 software.

Electron Microscopy

After necropsy, the left kidney cortex was immediately divided into pieces that were each 1 mm^3 and prepared for transmission electron microscopy as described by Seckin *et al.*[46] The samples were investigated by Jeol JEM 1011 transmission electron microscope.

Statistical Analysis The results were expressed as means ± standard error of the mean (S.E.M.). All statistical comparisons were performed by one-way ANOVA followed by Dunnett's tests. $p < 0.05$ was considered statistically significant.

CONCLUSION

To conclude, this is the first report which investigates the protective and ameliorative effects of *O. ficus indica* fruits against cisplatin-induced nephrotoxicity in mice. The pretreatment with cactus pear fruit extract significantly attenuated cisplatin-induced functional and histological renal deterioration. The obtained data displayed that antioxidant molecules such as quercetin, myricetin, luteolin can synergistically offer a renal protective function against cisplatin-induced nephrotoxicity.

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Conflict of Interest The authors declare no conflict of interest.

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