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Original Article

Effects of Fennel Essential Oil on Cisplatin-induced Nephrotoxicity in Ovariectomized Rats

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ABSTRACT

Background: Cisplatin (cis-diamminedichloroplatinum II (CDDP)) is an effective drug in cancer therapy to treat solid tumors. However, the drug is accompanied by nephrotoxicity. Previous reports indicated that estrogen has no protective role against CDDP-induced nephrotoxicity, but the role of phytoestrogen as an estrogenic agent in plants is not determined yet. The major composition of fennel essential oil (FEO) is trans-anethole that has estrogenic activity; so, we used FEO as a phytoestrogen source against CDDP-induced nephrotoxicity. **Materials and Methods:** Fifty-four ovariectomized Wistar rats were divided into seven groups. Groups 1-3 received different doses of FEO (250, 500, and 1000 mg/kg/day, respectively) for 10 days. Group 4 received saline for 10 days plus single dose of CDDP (7 mg/kg, intraperitoneally (ip)) at day 3. Groups 5-7 received FEO similar to groups 1-3, respectively; plus a single dose of CDDP (7 mg/kg, ip) on day 3. On day 10, the animals were sacrificed for histopathological studies. **Results:** The serum levels of blood urea nitrogen (BUN) and creatinine (Cr), kidney tissue damage score (KTDS), and kidney weight (KW) and body weight changes in CDDP-treated groups increased significantly ($P < 0.05$). FEO did not reduce the levels of BUN and Cr, KTDS, and KW and body weight changes. Also, the serum and tissue levels of nitrite were not altered significantly by FEO. **Conclusion:** FEO, as a source of phytoestrogen, did not induce kidney damage. In addition, FEO similar to estrogen was not a nephroprotectant agent against CDDP-induced nephrotoxicity.

Key words: Cisplatin, fennel essential oil, nephrotoxicity, ovariectomized rats

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II (CDDP)) is a chemotherapy drug widely used in clinic for treatment of solid tumors. One of the most important and common side effects of CDDP is nephrotoxicity.[1-4] The pathogenesis of CDDP-induced nephrotoxicity includes oxidative stress, inflammation, and necrosis in proximal tubule.[2,5] Many agents have been suggested to protect the kidney against CDDP-induced nephrotoxicity; including erythropoietin, vitamins E and C, L-arginine, N-acetylcysteine, and angiotensin receptor blocker–losartan.[5-11] It is also reported that CDDP-induced nephrotoxicity is gender-related[6] and pharmacological doses of estrogen could abolish the nephroprotectant effect of some antioxidants.[12] Although estrogen is well-known as a cardiovascular protectant in women before menopause, it is not nephroprotectant in CDDP-induced nephrotoxicity.[12,13] So, one question is raised here; whether phytoestrogen response to kidney

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toxicity is similar to estradiol. Genistein, a phytoestrogen source was used to prevent CDDP-induced renal injury in male rats, and decrease in inflammation and oxidative stress was reported.[5]

Phytoestrogens are estrogenic agents found in plants.[14-18] These agents may reduce the development of renal diseases.[17] Phytoestrogens also have estrogenic activity due to their structural resemblance to diethylstilbestrol, a synthetic estrogen.[19] *Foeniculum vulgare* Mill. (fennel) is an aromatic plant that is used in fresh, juice, or dried forms. It has been widely used as a flavoring agent in many dishes and salads.[19-21] Additionally, this plant have been used for treatment of some diseases such as dyspepsia, bronchitis, chronic cough, dysmenorrhea,[21] and also has been suggested to increase milk and libido in women.[20,22,23]

The major composition of fennel essential oil (FEO) is trans-anethole, fenchone, and limonene.[20] Among them, trans-anethole is the most important item, which has antispermatogenic effect and decreases sperm concentration in epidermis of male rats.[24] It is well-documented that trans-anethole has estrogenic activity and is a well-known phytoestrogen.[19-21,25]

Previously, we reported that estrogen itself is a risk factor to promote CDDP-induced nephrotoxicity in female,[12] however the role of phytoestrogen was not reported yet, and therefore in this study we investigate the protective effect of FEO on CDDP-induced nephrotoxicity in ovariectomized rats.

MATERIALS AND METHODS

Fifty-four adult female Wistar rats (weighting 183.5 ± 1.4 g) were used in this study. The animals were housed at the room temperature of 23-25°C. The rats had free access to water and chow. All procedures were approved by the Ethical Committee of Isfahan University of Medical Sciences.

Essential oil preparation

Foeniculum vulgare essential oil was kindly gifted from the Barij Essence Pharmaceutical Company, Mashhad Ardehal, Iran, in 2008.

Essential oil analysis

The gas chromatography (GC)/mass spectrophotometry (MS) analysis was performed on an Agilent 5975C Mass selective detector coupled with a Hewlett Packard 6890 gas chromatography equipped with a HP-5MS capillary column. The oven temperature was programmed from 60 to 280°C at the rate of 4°C/min. Helium was used as the carrier gas at the flow rate of 2 mL/min. The injector and detector temperature was 280°C. The MS operating parameters were: Ionization voltage 70 eV, ion source temperature 200°C. Identification of the natural constituents of the oil was based on retention indices relative to n-alkanes and computer matching with the WILEY 275.L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature [Table 1].[20,25,26]

Drug

CDDP (code P4394) was provided from Sigma (St. Louis, MO, USA).

Experimental protocol

The rats were ovariectomized as described previously.[3] After 5 days of recovery, the animals were randomly divided into seven experimental groups.

Groups 1-3 were considered as the negative control groups (n = 10 for each group), received 250, 500, and 1000 mg/kg/day FEO intraperitoneally (ip) for 10 days.

Group 4 (n = 4), as the positive control groups received saline for 10 days plus a single dose of CDDP (7 mg/kg, ip) on day 3.

Groups 5-7 received 250 (group 5, n = 6), 500 (group 6, n = 7), and 1000 mg/kg/day (group 7, n = 7) FEO for 10 days, plus a single dose of CDDP (7 mg/kg, ip) on day 3. The groups were considered as the cases groups.

The weight of animals was recorded daily. After 10 days of experiment, the rats were sacrificed after blood samples

Table 1: The list of the natural constituents of *F. vulgare* essential oil

Compound	Present	KI
Alpha-pinene	0.4	937
Camphene	0.1	951
Sabinene	0.9	970
Beta-pinene	0.6	978
Beta-myrcene	0.3	991
Alpha-phellandrene	0.1	1003
Para-cymene	0.7	1026
Limonene	3.5	1034
Cis-beta-ocimene	0.7	1039
Gamma-terpinene	0.5	1056
Fenchone	5.1	1079
Trans-sabinene hydrate	0.5	1086
Trans-limonene oxide	0.2	1124
Camphor	1.5	1133
Estragole	1.0	1189
Trans-anethole	74.0	1277
Alpha-copaene	0.2	1376
Germacrene-D	0.8	1482
Myristicin	0.4	1506

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were taken. The serum samples were collected and stored at −20°C until measurement. The kidney and uterus were removed immediately and weighed. The left kidney was fixed in 10% formalin for pathological investigation, and the right kidney was homogenated in 2 ml of saline; centrifuged at 15,000 rpm for 2 min; and the supernatant was collected for measurement.

Measurements

The levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were measured using diagnostic kits (Pars Azmoon Co., Tehran, Iran). The serum and renal levels of nitrite (NO stable metabolite) were determined by a commercial kit (Promega Corporation, Madison, WI, USA). The renal and serum levels of malondialdehyde (MDA) were measured by the manual method. Briefly, 0.5 mL of the sample was mixed by 1 mL of 10% trichloroacetic acid (TCA). The mixture was centrifuged at 2,000 g for 10 min; and 500 µl of the supernatant was added to 500 µl of 0.67% thiobarbituric acid (TBA) and was incubated in the boiling water for 10 min. After cooling, the absorbance was read at the wavelength of 532 nm.

Histopathological procedures

For histopathological investigation, the excised left kidney was embedded in paraffin and then stained by hematoxylin and eosin (H and E) to assay the tubular damage. The tubular damage was evaluated by an expert pathologist who was blind to the study with regard to tubular dilation and simplification, tubular cells swelling and necrosis, tubular casts, and intraluminal cell debris with inflammatory cells infiltration. According to intensity of tubular injuries, the kidney tissue damage score (KTDS) was assigned in the range of 1-4 by pathologist as blind, while zero was assigned to normal tissue without damage.

Statistical analysis

Data was expressed as mean ± standard error of the mean (SEM). Comparison of the groups with regard to body weight was performed using repeated measure analysis. The levels of BUN, Cr, MDA, and nitrite; and kidney weight (KW) were analyzed by the one-way ANOVA followed by the Tukey's test. The tissue damage score was compared by the Kruskal-Wallis or Mann-Whitney tests.

RESULTS

Effect of CDDP

Serum levels of BUN, Cr, and serum and tissue nitrite levels

The CDDP-induced nephrotoxicity was approved by comparison of BUN and Cr levels between the positive control group (group 4) and the negative control groups (groups 1, 2, and 3). The data indicated that the levels of BUN and Cr increased significantly in the positive control group ($P < 0.05$) [Figure 1]. The negative control groups were not significantly different in the BUN and Cr levels. Although the serum level of nitrite was not significantly different between groups 1-4, the kidney nitrite levels in negative control groups (FEO-alone treated groups) increased, and this increase was statistically significant in groups 1 and 3, when compared with the positive control group ($P < 0.05$) [Table 2].

Serum and tissue levels of MDA, body weight changes, KW, and KTDS

CDDP significantly increased the kidney tissue MDA level ($P < 0.05$), and such finding was not observed in the serum level of MDA [Table 2]. Both KW and KTDS increased significantly in the CDDP-alone treated (positive control) group compared with the negative control groups ($P < 0.05$) [Figure 1]. These findings verify CDDP-induced nephrotoxicity. No body weight loss was detected in the FEO-alone treated (negative control) group, but significant weight loss was observed in the CDDP-alone treated group ($P < 0.05$) [Figure 1].

Effect of FEO on CDDP-induced nephrotoxicity

Serum levels of BUN, Cr, and the serum and tissue nitrite levels

To determine the effect of FEO on nephrotoxicity induced by CDDP, all groups treated by the combination of CDDP and different doses of FEO were compared with the CDDP-alone treated group. The data obtained showed that FEO could not reduce the levels of BUN and Cr [Figure 2]. The results also indicated that the serum and tissue levels of nitrite did not significantly change by FEO [Table 3].

Serum and tissue levels of MDA, body weight changes, KW, and KTDS

The CDDP-received groups were not significantly different in serum and tissue levels of MDA. The KW and KTDS

Table 2: Levels of nitrite and MDA in serum and kidney in positive and negative control groups

Group	Serum nitrite (µmol/L)	Kidney nitrite (µmol/g tissue)	Serum MDA (µmol/L)	Kidney MDA (nmole/g tissue)
FEO (250 mg/kg), group 1	28.50±4.92	0.17±0.0*	3.71±1.19	2.01±0.48*
FEO (500 mg/kg), group 2	25.70±2.51	0.15±0.01	5.90±1.92	1.77±0.28*
FEO (1,000 mg/kg), group 3	19.76±3.07	0.22±0.02*	9.09±3.33	1.60±0.72*
CDDP +saline, group 4	23.08±3.11	0.09±0.01	6.19±1.18	5.01±1.14

MDA = Malondialdehyde, FEO = Fennel essential oil, CDDP = Cis-diamminedichloroplatinum II/cisplatin. *Significant difference from group 4, $P<0.05$

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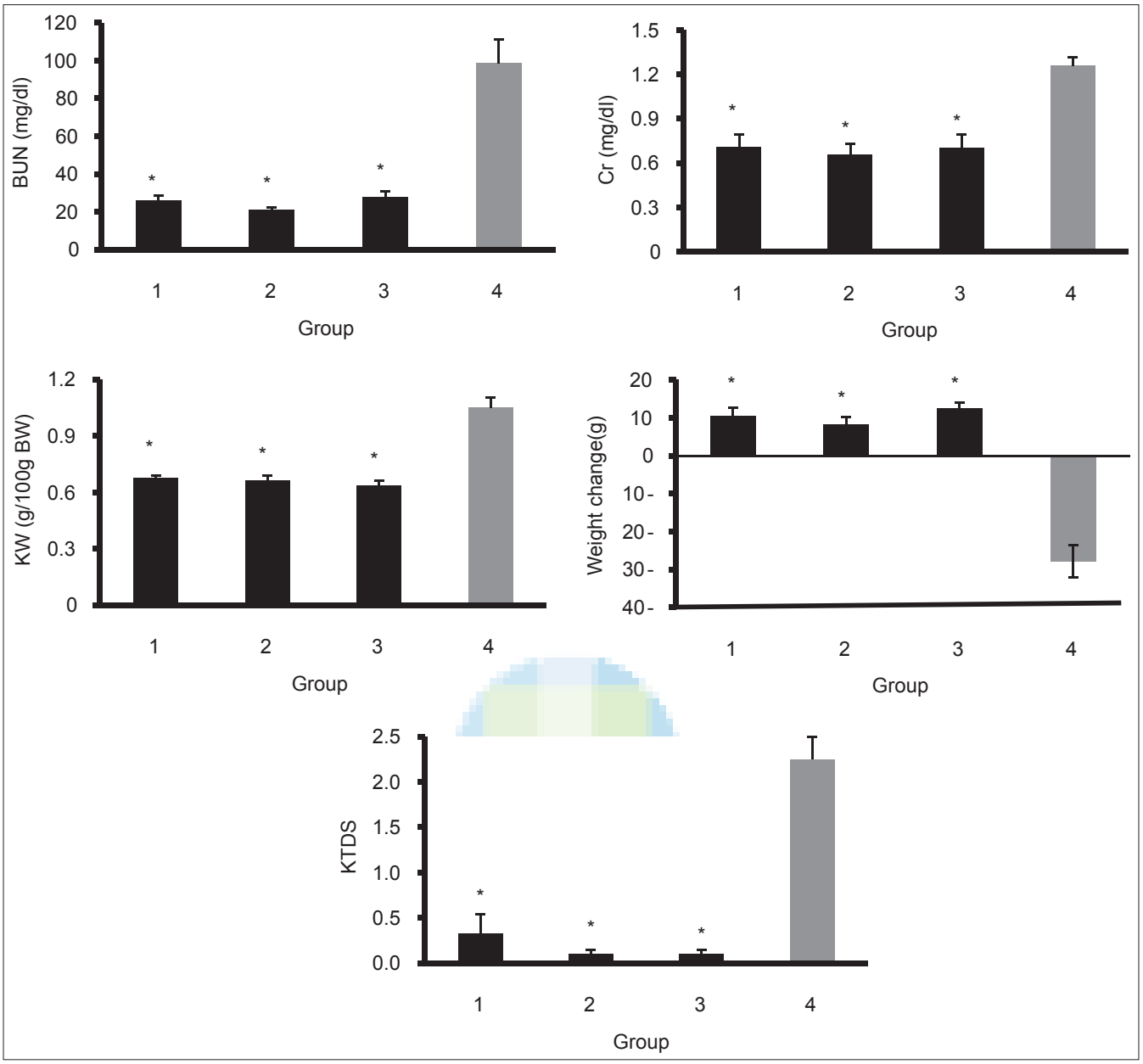


Figure 1: Serum levels of BUN and Cr; kidney weight; weight change and kidney tissue damage score in the positive (group 4) and negative control (groups 1, 2, and 3) groups. The star indicates significant difference from positive control group ($P < 0.05$)

increased, but the increase was not statistically significant between the positive control group (group 4) and the case groups (groups 5, 6, and 7). The weight loss in CDDP-alone treated group was more than that in the groups that received FEO plus CDDP. However, the weight loss in all groups that received CDDP was not statistically significant [Figures 2 and 3].

DISCUSSION

The main objective of this study was to determine the effect of FEO on CDDP-induced nephrotoxicity in ovariectomized rats. Our results indicate that CDDP increased the serum levels of BUN, Cr, and MDA. The KTDS and KW were also increased, and weight loss was observed in the presence of CDDP. This finding is in agreement with the results of other studies.[3,6,8,27-29]

CDDP resulted in significant tubular damage[28] and oxidative stress involved in kidney damage after CDDP administration.[30] The nephrotoxic effect of CDDP is related to its uptake by proximal tubular cells.[31,32] Some other studies documented that a single injection of CDDP at doses of 5-10 mg/kg in rats caused a marked reduction in the glomerular filtration rate and increase in serum levels of BUN and Cr; thus, indicating induction of acute kidney injury.[33-36] The weight loss in animals is caused

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Table 3: Serum and kidney levels of nitrite and MDA in positive control and case groups

Group	Serum nitrite (μmol/L)	Kidney nitrite (μmol/g tissue)	Serum MDA (μmol/L)	Kidney MDA (nmole/g tissue)
CDDP+saline, group 4	23.08±3.11	0.09±0.01	6.19±1.18	5.01±1.14
FEO (250 mg/kg) + CDDP, group 5	40.15±5.46	0.13±0.01	13.78±2.71	4.77±1.47
FEO (500 mg/kg) + CDDP, group 6	30.64±5.08	0.11±0.01	8.76±2.23	5.11±1.00
FEO (1,000 mg/kg) + CDDP, group 7	33.69±7.33	0.10±0.01	7.21±2.33	8.19±1.58

MDA = Malondialdehyde, FEO = Fennel essential oil, CDDP = Cis-diamminedichloroplatinum II/cisplatin

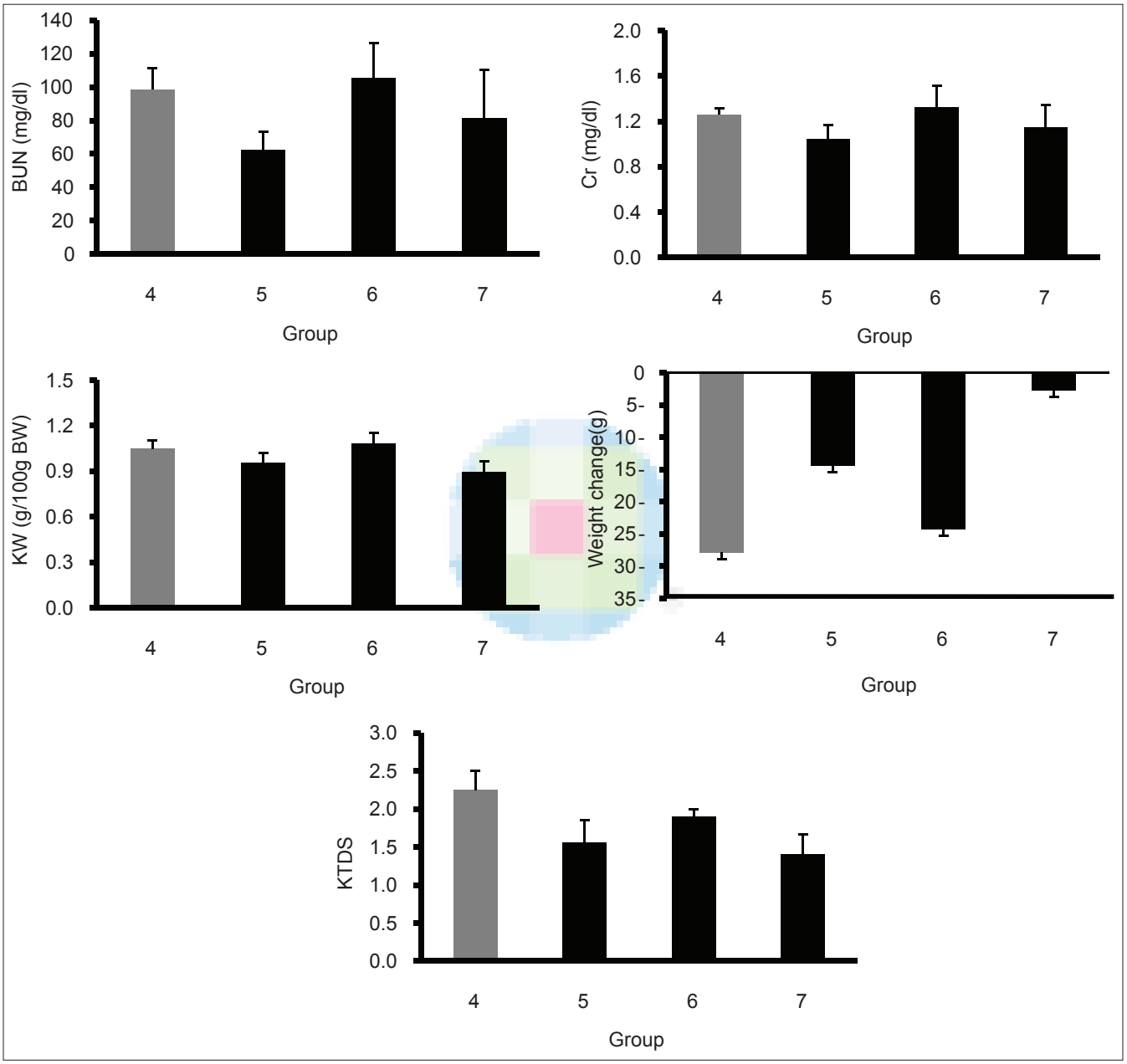


Figure 2: Serum levels of BUN and Cr; KW; weight change; and KTDS in positive control (group 4) and case (groups 5, 6, and 7) groups. The star indicates significant difference from positive control group ($P < 0.05$)

by CDDP-induced gastrointestinal disturbances.[6,37-39] We observed that KW increased by CDDP, possibly due to tubular damages and changes in glomerular filtration rate,[40] which accumulates water and salt in the kidney tissue. CDDP induced free radical protection and lipid peroxidation in tubular cells that is probably responsible for the oxidative renal damage. Lipid peroxidation was monitored by measuring MDA, which results from free radical damage to the membrane components of the cells.[28,29]

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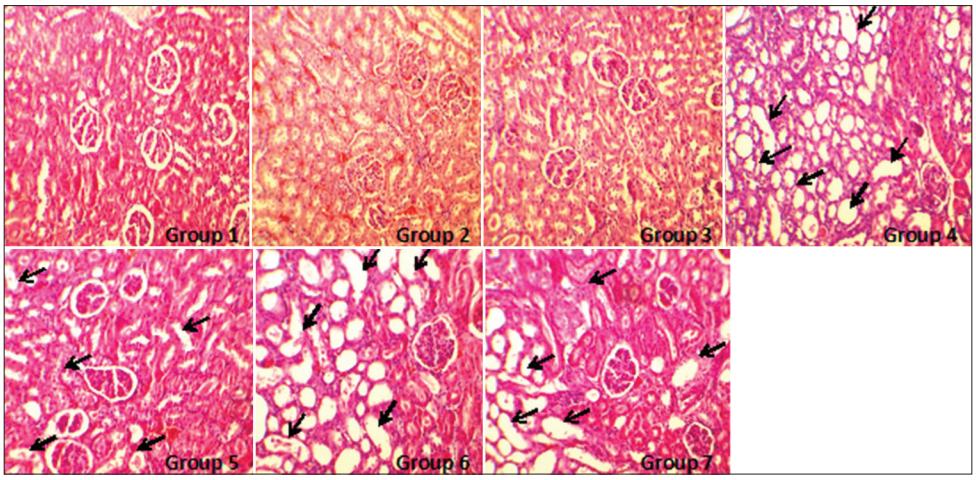


Figure 3: The images of kidney tissues (magnification, ×100) in all groups of experiments. The damage was detected by tubular dilation and simplification, tubular cells swelling and necrosis, tubular casts, and intraluminal cell debris with inflammatory cells infiltration. More kidney tissue damage was observed in groups 4-7 (with no significant difference between the groups) while no damage was detected in the groups 1-3

According to our results, FEO has not protective role against nephrotoxicity. The major component of FEO is trans-anethole that has estrogenic activity.[20,21,41] It seems that tarns-anethole have the potential to interact with estrogen receptors.[20] Estrogen can improve the potency of CDDP[42] and promote the kidney damage.[12,13] Furthermore, estrogen has vasodilatory effect and more CDDP may be transported to the kidney, and CDDP accumulates in the kidney tissue via basolateral membranes and destroys mitochondrial DNA, and finally causes tubular damage.[40,43,44] Moreover, studies showed that estrogen increases oxidative stress in kidney[12,45] and promotes kidney toxicity in proximal tubules.[6,12] Therefore, it seems that the effect of FEO on CDDP-induced nephrotoxicity is very similar to synthetic estrogen which is not protecting CDDP induced kidney toxicity, possibly different mechanism was involved. CDDP enhances the level of renal inducible NO synthase (iNOS) in response to oxidative stress;[28,45,46] but in our previous studies, CDDP did not cause any changes in nitrite levels.[12,13] FEO is a source of NO, especially iNOS.[47] So, increase in the NO kidney level in negative control groups is probably caused by NO. But, the level of kidney NO decreased when CDDP was injected. Therefore, positive control group and case groups were not significantly different in this regard. This result is probably due to the effect of CDDP on reduction of endothelial NO[12] and it possibly prevents the protective effect of FEO on CDDP-induced nephrotoxicity. On the other hand, studies have shown that the FEO effect is dose dependent;[20] so, probably doses used in the current study were not effective doses.

CONCLUSIONS

It seems that FEO, as a source of phytoestrogen,[20,41,48] did not induce nephrotoxicity. In addition, FEO was not a nephroprotectant agent against CDDP-induced nephrotoxicity.

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