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The effect of white tea on serum TNF-α/NF-κB and immunohistochemical parameters in cisplatin-related renal dysfunction in female rats☆

Sinan Saral[a], Eda Dokumacioglu[b], Tolga Mercantepe[c], Mehtap Atak[d], Seda Cinar[c], Ozlem Saral[e], Lamiye Yildiz[a], Hatice Iskender[b,*], Levent Tumkaya[c]

[a] Department of Physiology, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey
[b] Department of Nutrition and Dietetics, Faculty of Health Sciences, Artvin Coruh University, Artvin, Turkey
[c] Department of Histology and Embryology, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey
[d] Department of Biochemistry, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey
[e] Department of Nutrition and Dietetics, Health High School, Recep Tayyip Erdogan University, Rize, Turkey

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ABSTRACT

Objective: Nephrotoxicity is the most important side effect of the antineoplastic drug cisplatin, thereby restricting its use. The aim of this study was to investigate the protective effects of white tea infusions (WT) against renal damage induced by cisplatin (CP) in rats by biochemical and histopathological means.

Materials and methods: This study used 24 female Sprague Dawley rats at 12–14 weeks of age and weighing 250–300 g. Rats were divided into three groups: Control, CP and CP + WT groups. CP was injected 7 mg/kg i.p as a single dose/rat in the CP group. White tea was given at a dose of 0.5% (w/v) for 4 weeks. At the end of the experiment, blood urea nitrogen (BUN), creatinine, uric acid, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and nuclear factor kappa B (NF-κB) along with caspase-3 in the kidney were evaluated in study.

Results: BUN, creatinine, TNF-α, NF-κB and IL-6 levels of the CP group showed a statisically significant increase in comparison to the control group. TNF-α, NF-κB and IL-6 levels showed a statistically significant decrease in the CP + WT group with respect to the CP group. Caspase-3 levels in tubular epithelial cells decreased in CP + WT group compared with CP group ($p = 0.02$).

Conclusion: White tea infusions reduced significantly the nephrotoxicity of CP. The anti-nephrotoxic feature of the infusion may be attributed primarily to its anti-inflammatory and anti-apoptotic characteristics.

1. Introduction

Nephrotoxicity induced by antineoplastic drugs is one of the most common side effects of chemotherapy. Antimetabolites, alkylating agents and anthracyclines that are used in the treatment of cancer are the foremost agents causing to nephrotoxicity [1]. These chemotherapeutic agents may cause renal damage and dysfunction primarily in the three main parts of the nephron; the proximal tubule, the distal tubule, and the glomerulus. Additional severe consequences may occur such as serum electrolyte disturbances, increased levels of serum creatinine, reduced glomerular filtration rate (GFR), and permanent renal failure [2]. Cisplatin (CP) mediates anti-tumoral effects through a number of different cytotoxic mechanisms. CP binds to nuclear DNA and interferes with normal transcription, and/or DNA replication. CP induces apoptosis by increasing mitochondrial permeability through various mechanisms. However, this apoptotic effect plays a role in CP toxicity as well [3,4]. In addition to DNA damage, dysfunction of the cytoplasmic organelles, cellular damage through oxidative stress and inflammation are among the consequences of CP toxicity [5,6].

Nephrotoxicity is the most important side effect of CP, restricting its use [5]. In a study, nephrotoxicity was observed in approximately 1/3 of cancer patients treated with CP [5]. Nephrotoxicity begins to develop ten days after CP administration, with a clinical picture involving a decrease in glomerular filtration rate, increase in serum creatinine, and decrease in serum magnesium and potassium [7,8]. It is of critical importance to clarify the underlying mechanisms of CP-associated nephrotoxicity. Understanding these mechanisms could help achieve renoprotection without suppressing its antitumor effect.

Recent studies revealed the important role of the inflammatory response in cisplatin toxicity. CP increases several inflammatory

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* Corresponding author at: Department of Nutrition and Dietetics, Faculty of Health Sciences, Artvin Coruh University, Artvin, Turkey.
E-mail address: haticeiskender2011@hotmail.com (H. Iskender).

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Table 1
Ground white tea analysis by HPLC and AAS.

Component	Analysis method	Result	Unit
Gallic acid	ISO 14502-2/2005	0.05 (Dry matter)	%
Epigallocatechin (EGC)	ISO 14502-2/2005	0.33 (Dry matter)	%
Catechin (C)	ISO 14502-2/2005	0.00 (Dry matter)	%
Caffeine	ISO 14502-2/2005	4.38 (Dry matter)	%
Epicatechin (EC)	ISO 14502-2/2005	0.14 (Dry matter)	%
Epigallocatechin gallate (EGCG)	ISO 14502-2/2005	6.98 (Dry matter)	%
Epicatechin gallate (ECG)	ISO 14502-2/2005	2.52 (Dry matter)	%
Total polyphenol analysis	ISO 14502-2/2005	14.98 (Dry matter)	%
P	Spectrophotometric method	0.64	%
Cu	KACAR B,1991	16.37	ppm
Zn	KACAR B,1991	46.29	ppm
Fe	KACAR B,1991	57.75	ppm
Mn	KACAR B,1991	309.62	ppm
Mg	KACAR B,1991	1001.07	ppm
Na	KACAR B,1991	57.76	ppm
K	KACAR B,1991	13942.13	ppm
Al	KACAR B,1991	241.50	ppm
Ca	KACAR B,1991	2323.33	ppm

HPLC: high performance liquid chromatography, AAS: atomic absorption spectroscopy.

cytokines and chemokines by causing the nuclear factor-kappa B (NF-κB) translocation to the nucleus [9,10]. Additionally, inflammatory cytokines and chemokines were shown to increase CP cytotoxicity [11]. TNF-α, belongs to the TNF superfamily and is mainly produced by monocytes and macrophages, in response to various inflammatory and immunomodulatory stimuli. TNF-α has a very broad range of bioactivity, and most cells are sensitive to TNF-α. Under normal physiological conditions, TNF-α is involved in the formation of an immune response, cellular homeostasis, cell survival, proliferation, migration and differentiation [12,13]. Interleukin 6 (IL-6), another pro-inflammatory cytokine, is involved in many physiological and pathological events, playing a role in the immune response, hepatic acute phase response, hematopoiesis, regulation of neuronal functions and osteoclast formation. It is thought that IL-6 may have an important role in the pathogenesis of many diseases [14].

NF-κB is an important regulator of numerous genes responsible for inflammation, lymphocyte activation, cell growth and apoptosis [15]. NF-κB is involved in the cellular response to stimuli such as inflammatory cytokines, free radicals, carcinogens, chemotherapeutics, bacterial and viral agents. NF-κB, is also important in the pathophysiology of many diseases due to inflammatory cell damage [16].

In cancer therapy, the focus is on reducing or eliminating the side effects of the drugs in use. For this reason, various substances with high antioxidant activity are under investigation. Tea, containing more than 4000 chemical compounds, is one of the plants with the highest flavonoid content with respect to its dry weight. Particularly the flavonoids in tea were shown to enhance resistance to various diseases in epidemiological studies. In addition, tea has antioxidative, antimutagenic, anticarcinogenic, and anti-inflammatory effects due to catechins [17,18]. White tea (*Camellia sinensis L.*) is obtained from young tealeaves and buds. It has a higher antioxidant activity with respect to black tea and green tea because it is rich in catechin and is minimally oxidized during production [19]. The epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC) and epigallocatechin gallate (EGCG) present in high quantitites in WT contribute significantly to its bioactivities [20,21].

The aim of this study was to investigate the protective effects of WT on renal damage induced by CP in rats, using biochemical and histopathologic means. From this point of view, blood urea nitrogen (BUN), creatinine, uric acid, TNF-α, IL-6 and NF-κB levels in serum samples and caspase-3 in kidney tissue samples were evaluated in our study.

2. Materials and methods

2.1. Chemicals

Cisplatin was obtained from Faulding Pharmaceuticals Plc (Warwickshire, UK). The other chemicals used in the study were obtained from Sigma (Sigma Chemicals Co., St. Louis, MO, USA).

2.2. Experimental animals and study groups

Twenty four female Sprague Dawley rats (12 ± 2 weeks of age and weighing 278.79 ± 11.10 g) were used in this study. Experimental animals were obtained from the Laboratory Animals Research Center of Recep Tayyip Erdoğan University. Ethical approval for this study (2018/4) was obtained from the Local Ethics Committee for Laboratory Animals of Recep Tayyip Erdoğan University. For adaptation, the rats were kept in air-conditioned room with a temperature of 23 ± 2 °C, a humidity of 55 ± 5% and 12 h of light/dark cyclesfor 1 week, before the initiation of the study. All animals had free access to food and water. The animals were alocated into 3 groups, with 8 members each. The control group kept untreated. The CP and WT + CP groups were injected intraperitoneally with cysplatin as a single dose of 7 mg/kg/rat, sufficient to induce nephrotoxicity [22,23]. Before CP administration, however, the WT + CP group had been given 0.5% w/v WT in drinking water prepared daily for 4 weeks.

2.3. Provision and preparation of white tea samples

White tea samples were obtained from manufacturer (General Director of Tea Enterprises, Rize, TURKEY). The phenolic contents analysed by high performance liquid chromatography (HPLC; ISO 9002 standard) [24] and the mineral composition deterrmined by 'Kacar B analysis method [25] of WT are listed in Table 1. The WT infusion was prepared by a modification of Nunes AR (2015). Briefly, WT was added 100 °C boiled water (0.5 g/100 ml) and infused for 3 min. Then, the infusion was filtered through a 0.2 μM cellulose acetate filter. The volume was made up to 100 ml by adding water. The infusions were prepared daily and cooled to room temperature before administration to rats [26].

2.4. Preparation of blood and tissue specimens

At the end of the study, blood and renal tissue samples were taken, following 12 h of fasting, by opening the abdominal and thoracic

cavities of rats under general anesthesia with ketamine-xylazine. Blood samples were collected into tubes containing no anticoagulants, kept at RT for a few minutes, then centrifuged at 4000 × *g* for 15 min at + 4 °C, and thus obtained serum were stored at − 20 °C until analysis for BUN, creatinine, uric acid, TNF-α, NF-κB and IL-6. Kidney tissue samples taken from rats were rinsed three times with cold (4 °C) 0.9% sodium chloride. Those kidney tissue samples allocated for histopathological analysis were taken into neutral formalin and stored at − 80 °C until analysis. Caspase-3 activities were determined immunohistochemically in kidney tissue specimens.

2.5. Biochemical analysis

TNF-α, NF-κB and IL-6 levels were determined in serum samples by ELISA (SunRed Biological Technology, Co., Ltd. Shanghai) following the manufacturer's procedure. The NF-κB levels were stated as ng/ml while those of TNF-α, and IL-6 were stated as pg/ml. Biochemical tests for blood urea nitrogen (BUN), creatinine and uric acid were performed using the Abbott Architect®c16000 analyzer (Abbott Diagnostics, Abbott Park, IL, USA).

2.6. Histopathological analysis

Kidney tissue were fixed for 48 h in 10% formalin (Sigma Aldrich, St. Louis, MO, USA). Following the fixation procedure, tissue specimens were passed through routine histological procedures and embedded in paraffin blocks (Merck, Darmstadt, Germany). 3 − 4 μm sections were taken using a microtome (Leica, RM2125RT, Germany) and stained with Harris hematoxylin and Eosin G (Merck, Darmstadt, Germany). The sections were then examined under a light microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan). Photographs were taken using an Olympus DP71 (Olympus Corporation, Tokyo, Japan) digital camera.

2.7. Semi-quantitative analysis

The tubular necrosis score (TNS) method developed by Sung et al. [27] was used during histopathological examination of kidney sections (Table 2). Semi-quantitative analysis was performed blindly by two independent histopathologists (TM and LT). The histopathologists were aware of the contents of the various study groups up to tissue processing procedures. The researcher classifying the histopathological sections was also blinded to the treatment groups.

Table 2
Tubular necrosis scoring method.

Score	%
Deterioration of brush border structure in proximal tubules	
0	No damage
1	≤10%
2	10–25%
3	26–75%
4	≥75%
Apical membrane blebbing	
0	No damage
1	≤10%
2	10–25%
3	26–75%
4	≥75%
Loss of tubular epithelial cell connections (debris accumulation in the lumen)	
0	No damage
1	≤10%
2	10–25%
3	26–75%
4	≥75%

2.8. Quantitative analysis

Renal corpuscle and proximal tubule surface areas were measured with an Olympus DP2-BSW (Ver.2.1 to Ver.2.2, Build 6212, Tokyo, Japan) software operated polygonal probe. Renal corpuscle and proximal tubule surface areas were measured at x20 and x40 magnifications, respectively. Measurement margins for H&E-stained sections were determined by two independent histopathologists (TM and LT) [28].

2.9. Immunohistochemical (IHC) analysis procedure

3 − 4 μm sections taken from the paraffin blocks were placed onto positively charged slides. They were kept for 10 min in 3% H2O2 solution. The sections were next washed in phosphate buffer solution (PBS) and incubated with citrate buffer solution for 5 min. Subsequently, they were blocked for 15 min in secondary blocking solution. They were then incubated with primary antibody (Caspase-3, Rabbit polyclonal to Caspase-3, 1/100, ab4051, Abcam, UK) and secondary antibody (EXPOSE Rabbit specific HRP/DAB detection IHC Kit, ab80437, Abcam, UK) for 60 min. Finally, sections stained with Harris hematoxylin and diaminobenzidine chromogen solution were covered with entellan (Superior Mansfield, Germany). Grading of the caspase-3 staining score is presented in Table 3.

2.10. Statistical analysis

All data were analysed using SPSS 18.0 (IBM, Armonk, NJ, USA) software. Non-parametric data were calculated as median ± standard deviation based on the minimum and maximum values. Differences between groups were analyzed using the Kruskal Wallis test, followed by Tamhane's test, and the groups' numerical data were then subjected to analysis, with p values < 0.05 being regarded as significant. Parametric data were calculated as mean ± S.D. Differences between groups were analyzed using One-Way ANOVA followed by the Tukey HSD test, and groups' numerical data were then subjected to analysis, with p values < 0.05 being regarded as significant.

3. Results

3.1. Biochemical results

BUN, uric acid, and creatinine values of the study groups are presented in Table 4. BUN and creatinine levels of the CP group showed a statistically significant increase in comparison to the control group ($p < 0.05$). There was a mild increase in the uric acid levels of the CP group, which was not a statistically insignificant ($p > 0.05$). BUN, uric acid and creatinine levels showed a statistically significant decrease in the CP + WT group with respect to the CP group ($p < 0.05$, $p < 0.001$, $p < 0.05$, respectively). TNF-α levels, however, showed a statistically significant increase in the CP group with respect to the control group (Fig. 1) ($p < 0.001$). TNF-α levels of the CP + WT group showed a significant decrease when compared to the CP group ($p < 0.05$). TNF-α levels of the CP + WT group were similar to those of the control group. There was no statistically significant difference between serum TNF-α levels of the CP + WT and the control groups ($p > 0.05$). Serum levels of NF-κB (Fig. 2) and IL-6 (Fig. 3) were statistically significantly increased in the CP group with respect to the

Table 3
Grading of caspase-3 staining intensity.

0	None (less than 5%)
1	Mild (less than 25%)
2	Moderate (involvement 25%–50%)
3	Severe (more than %50)

Table 4
Serum biochemical parameters.

	Control	CP	CP + WT
BUN (mg/dL)	36.3 ± 8.97	163.00 ± 76.80[a]	33.80 ± 4.64[b]
Creatinin (mg/dL)	0.58 ± 0.08	1.67 ± 0.59[a]	0.61 ± 0.07[b]
Uric acid (mg/dL)	1.40 ± 0.18	1.62 ± 0.25	1.08 ± 0.40[c]

Data are given as mean ± SD.
BUN: Blood Urea Nitrogen, CP: Cisplatin, WT: White Tea.
[a] Significantly different when compared with the control group, ($p < 0.05$).
[b] Significantly different when compared with the CP group, ($p < 0.05$).
[c] Significantly different when compared with the CP group, ($p < 0.001$).

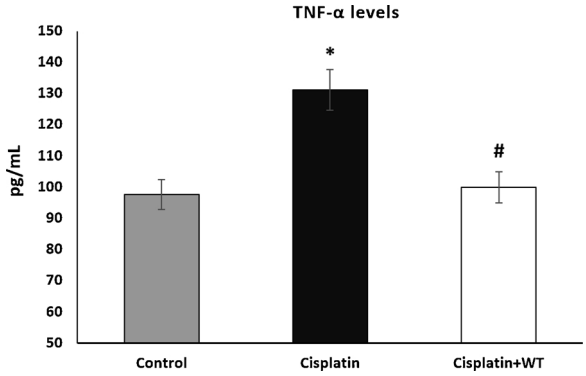


Fig. 1. Effect of white tea on serum TNF-α levels in rats with cisplatin-induced nephrotoxicity.
Data represent mean ± SEM (n = 8 per group). *P < 0.001 compared with the control group; #P < 0.05 compared with the cisplatin group. Abbreviations: WT-white tea.

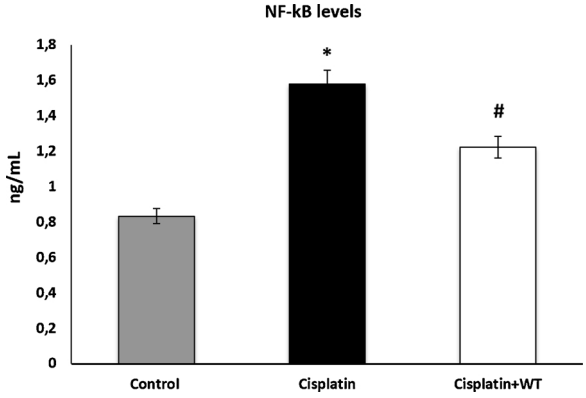


Fig. 2. Effect of white tea on serum NF-κB levels in rats with cisplatin-induced nephrotoxicity. Data represent mean ± SEM (n = 8 per group). *P < 0.05 compared with the control group; #P < 0.05 compared with the cisplatin group. Abbreviations: WT-white tea.

control group ($p < 0.05$). In contrast, CP + WT group had a statistically significant decrease in NF-κB and IL-6 levels than the CP group ($p < 0.05$). There was no significant difference in terms of NF-κB and IL-6 levels between the CP + WT group and the control group ($p > 0.05$).

3.2. Histopathological results

Sections from the control exhibited a normal histological structure in the cortex and medulla (median TNS score: 1.00 ± 0.52; Table 5; Fig. 4A–B). Sections from the cisplatin group, displayed necrotic structure in epithelial cells of the proximal and distal tubules (Fig. 4C–D; Table 5; median TNS value: 9.00 ± 0.75). Cast formation was observed together with tubular necrosis in the proximal and distal

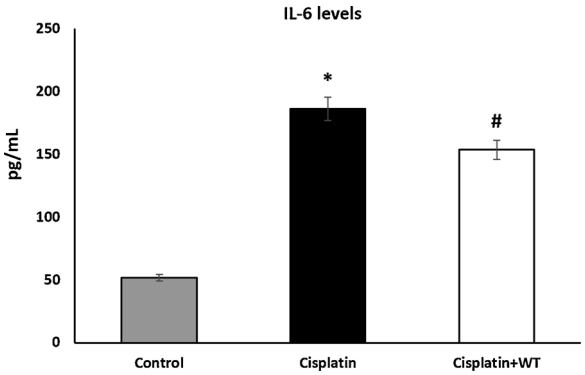


Fig. 3. Effect of white tea on serum IL-6 levels in rats with cisplatin-induced nephrotoxicity.
Data represent mean ± SEM (n = 8 per group). *P < 0.05 compared with the control group; #P < 0.05 compared with the cisplatin group. Abbreviations: WT-white tea.

Table 5
TNS score analysis.

	TNS	Brush border damage score	Apical membrane blebbing score	Luminal Debris accumulation score
Control	1.00 ± 0.52	0.00 ± 0.52	3.00 ± 0.52	0.00 ± 0.52
Cisplatin	9.00 ± 0.75[a]	3.00 ± 0.63[c]	3.00 ± 0.41[e]	3.00 ± 0.52[g]
Cisplatin + WT	1.00 ± 1.60[b]	1.00 ± 0.41[d]	1.00 ± 0.52[f]	1.00 ± 0.52[h]

*Median ± Standart Deviation.
Kruskal Wallis -Tamhane's T2 test.
[a] $P = 0.00$ versus Control group.
[b] $P = 0.364$ versus Cisplatin group.
[c] $P = 0.00$ versus Control group.
[d] P = 0.01versus Cisplatin group.
[e] $P = 0.00$ versus Control group.
[f] P = 0.00 versus Cisplatin group.
[g] P = 0.00 versus Control group.
[h] P = 0.00 versus Cisplatin group.

tubules (Fig. 4C–D; Table 5; median TNS value: 9.00 ± 0.75). Pronounced areas of infiltration were also evident in peritubular areas (Fig. 4C–D). In kidney sections from the WT-CP groups, the proximal tubules exhibited normal histological architecture (Fig. 4E–F; Table 5; median TNS value: 1.00 ± 1.60). Additionally, the brush borders in the proximal tubules also exhibited a typical architecture (Fig. 4E–F).

3.3. Semi-quantitative results

Cisplatin resulted in an increase in TNS compared to the control group (p = 0.00; Table 5). However, it reduced TNS in the WT + CP treatment group (p = 0.00; Table 5).

3.4. Immunohistochemical results

Tubular cells in the control group exhibited a normal appearance (Fig. 5A–B; Table 6). On the other hand, the number of apoptotic cells exhibiting caspase-3 positivity was higher in kidney tissue sections from the CP than in the control group (p = 0.00; Fig. 5C–D; Table 6). In the WT + CP group, the number of tubular epithelial cells exhibiting caspase-3 positivity decreased significantly (p = 0.02; Fig. 5E–F; Table 6).

3.5. Quantitative results

CP sections revealed significant decreases in renal corpuscle and proximal tubule surface areas compared to the control group (p = 0.00;

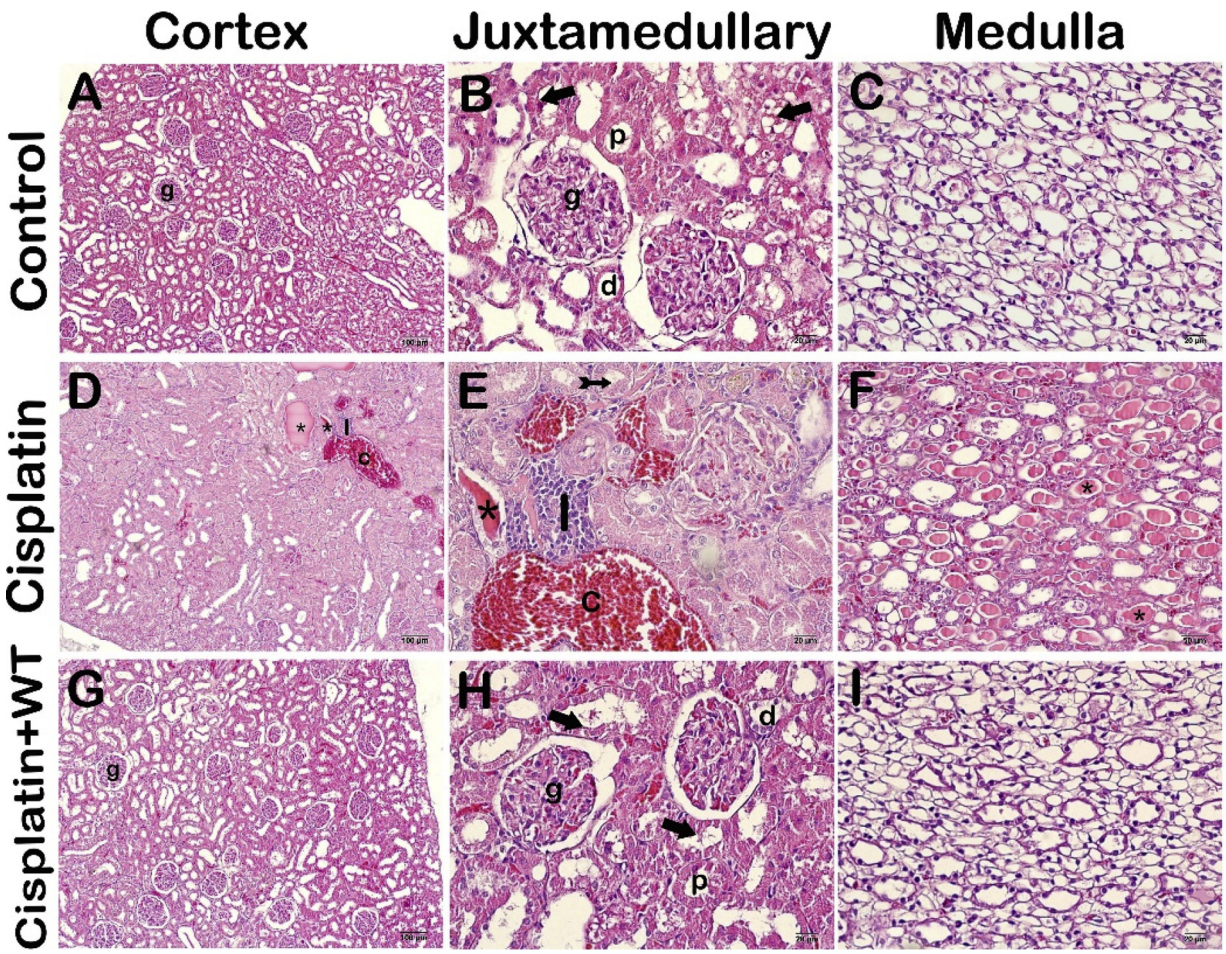


Fig. 4. Representative photographs of renal tissue sections treated with WT on renal histology in CP-induced renal injury. H&E staining.
Proximal tubule (p). Distal tubule (d). Glomerule (g). Brush border (arrow).
A(x10)-B(x40)-C(x40): Section from control group show normal glomerule (g), proximal (p) and distal tubules (d) with a normal structure in sections from the control group. Besides, the brush border structure (arrow) is clearly visible in the proximal tubules (median TNS score: 1.00 ± 0.52).
C(x10)-D(x20)-E(x20): Cisplatin group section shows a tubular necrosis. Numerous proximal tubules epithelial cells (arrow) have lost their brush borders and debris accumulation in the tubular lumen (asterisk) observed. Leukocyte infiltration can be seen in intertubular areas and vascular congestion (C) (median TNS value: 9.00 ± 0.75).
G(x20)-H(x40)-I(x40): Sections from the WT treatment group show typical glomerules (g), distal (d) and proximal tubules (p) with a brush border (median TNS value: 1.00 ± 1.60).

Table 7). However, we observed increases in renal corpuscle and proximal tubule surface areas in the WT + CP group compared to the CP group ($p = 0.00$; Table 7).

4. Discussion

CP use results in renal toxicity that can damage human kidney functions in a dose-dependent manner as assessed by serum BUN and creatinine levels [29–34]. In this study, we showed that serum TNFα, IL-6, NF-κB, BUN and creatinine levels were higher in CP treated rats than untreated controls, in addition to the higher intensity staining for caspase 3. In contrast, in WT treated rats, the levels of these parameters decreased significantly.

Recent studies focused on the use of anti-oxidants like flavonoids to counteract CP-induced cytotoxicity In our study, serum BUN and creatinine levels significantly decreased in the CP + WT group in comparison to the CP group, at levels close to the control group. White tea, containing higher concentrations of Polyphenolic catechin with respect to green tea and black tea, became very popular and highly preferred in recent years. Its antioxidant activity is more preserved because it is prepared without blanching, roasting and fermentation procedures. Studies concerning the use of WT in the literature are very few. Ahmad et al. [31] established CP-induced renal damage in rats, which was prevented by prior administration of black tea. Ahn et al. [35] observed a similar effect with green tea given before CP.

In this study, we aimed to determine the protective efficacy of WT against CP-induced nephrotoxicity because of its higher polyphenolic (mainly EGCG) contents. The exact mechanisms of CP-induced nephrotoxicity are yet to be determined but an ensuing inflammatory reaction resulting in pathogenesis and dysfunction may be common to some, if not all. In this regard, various mediators related to inflammation were investigated [5].

TNF-α is a cytokine with an essential role in inflammation, released by macrophages and lymphocytes, and suppressing the proliferation of tumor cells. Moreover, it has important effects on cell differentiation, apoptosis, natural and acquired immunity. One of the most important features of TNF-α is that it shows cytotoxic effects on tumor cells but not in normal cells [36,37]. In their study on rats, Kelly et al. [38] reported increased levels of TNF-α in kidneys that developed CP nephrotoxicity. In another study, Deng et al. [39] found an association between elevated TNF-α levels and acute CP-triggered renal injury in rats. In a study conducted by Ramesh et al. [13], increased levels of TNF-α were reported in the CP-induced nephrotoxicity model, while TNF-α inhibitors decreased renal damage.

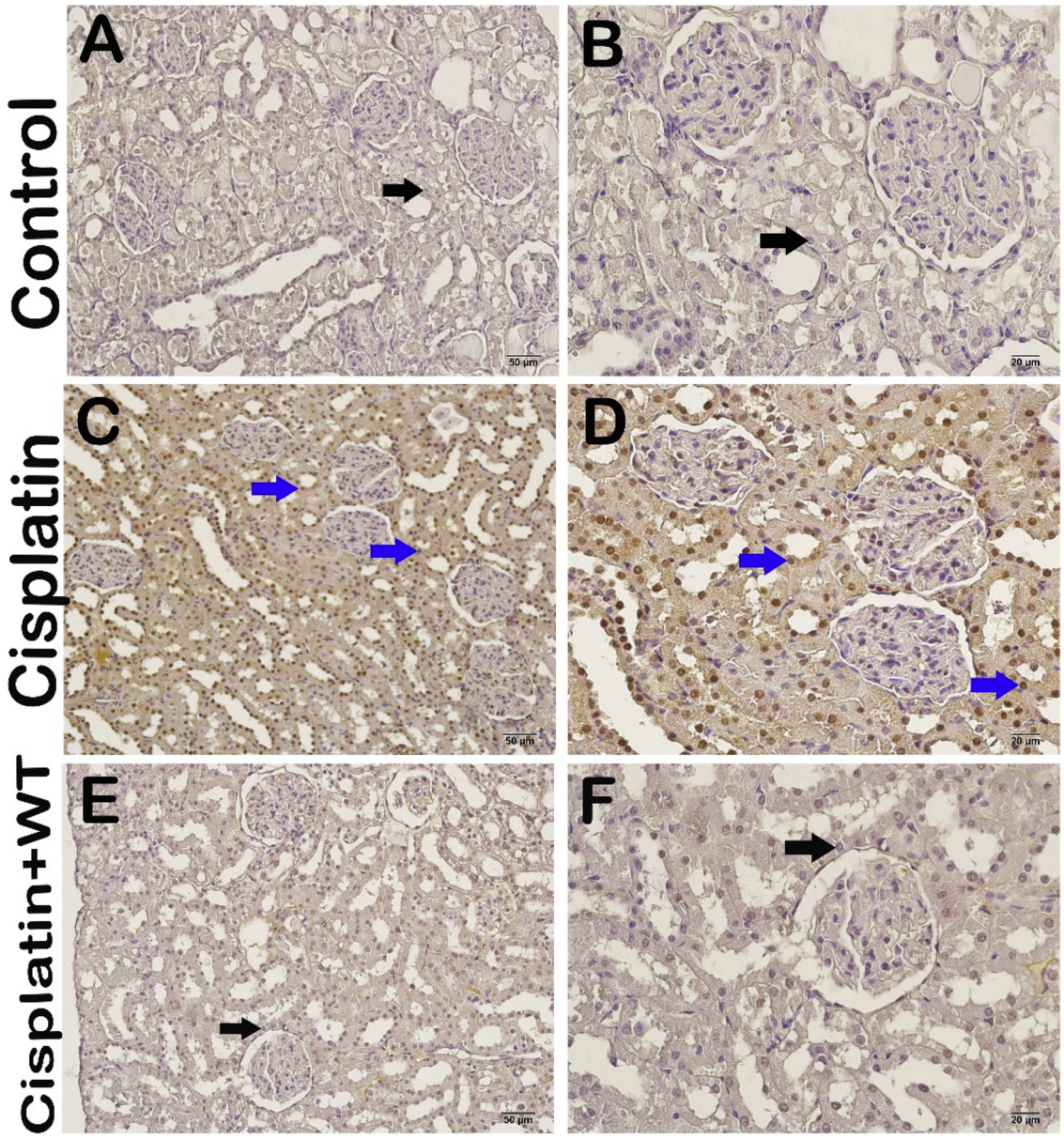


Fig. 5. Light microscopy image of the effect of WT treatment on apoptotic changes after one dose CP administration. (Arrows indicate Caspase-3 staining).
A(x20)- B(x40): Control group sections show normal tubular epithelial cell.
C(x20)- D(x40): Cisplatin group sections show apoptotic epithelial tubule cells. **E(x20)- F(x40):** WT treatment group show typical tubular epithelial cell.

Table 6
Caspase-3 positivity score.

Group	Caspase-3 positive score
Control	0.00 ± 0.41
Cisplatin	3.0 ± 0.52[a]
Cisplatin + WT	1.00 ± 0.63[b]

Kruskal Wallis -Tamhane's T2 test.
[a] $P = 0.00$ versus control group.
[b] $P = 0.02$ versus cisplatin group.

Table 7
Areas of renal corpuscle and proximal tubules (μm^2).

Group	Renal corpuscle area	Proximal tubules area measurement
Control	388.81 ± 26.76	388.81 ± 26.76
Cisplatin	174.66 ± 21.20[a]	172.91 ± 12.04[c]
Cisplatin + WT	380.26 ± 34.34[b]	369.44 ± 32.28[d]

Kruskal Wallis -Tamhane's T2 test.
[a] $P = 0.00$ versus control group.
[b] $P = 0.00$ versus cisplatin group.
[c] $P = 0.00$ versus control group.
[d] $P = 0.00$ versus cisplatin group.

In our study, whose findings are compatible with those in the literature, serum TNF-α levels of the CP group significantly increased in comparison to the control group. IL-6 is produced as a response to TNF-α and IL-1, which play a key role in the development of immunological and inflammatory events [40]. In the study by Yousef and Hussien [41], renal damage was induced in rats with CP, and increased levels of TNF-α and IL-6 levels were reported. Kumar et al. [42] induced renal damage in rats using 7,5 mg/kg CP, and reported increased levels of TNF-α and IL-6 in kidney tissue specimens. Based on our results and those of the relevant literature, it is evident that inflammation is an important step in the formation of nephrotoxicity. We think that increased expression of inflammatory mediators increase the degree of

renal damage and inhibition of these mediators may halt or prevent this. It appears, therefore, that measurement of IL-6 and TNF-α levels is important for the diagnosis and follow-up of nephrotoxicity.

It is apparent that numerous factors and various signaling pathways are involved in the etiology of CP-induced nephrotoxicity. However, NF-κB-mediated inflammatory response has gained prominence in recent years [43]. NF-kB is effective in a wider context such as immunity, inflammation, cell development, cell growth, maintenance of vital activities, and apoptosis [44]. In addition to its inflammatory effects, its anti-inflammatory effects, mediated by the activation of various anti-inflammatory cytokines or the inhibition of pro-inflammatory gene expressions were also reported. NF-κB can induce leukocyte apoptosis in certain conditions and contribute to the inhibition of inflammation, and therefore to the negative feedback control of inflammation [45].

In our study, NF-κB levels showed a significant increase in the CP group in comparison to the control group, and this increase was in parallel with TNF-α and IL-6 levels. Elseweidy et al. [46] reported that NF-κB levels increased in the rats given CP. Nevertheless, in the same study, they indicated a significant correlation between NF-κB levels and serum urea and creatinine levels. Again, another study reported increased NF-κB levels in CP-administered rats [47]. It is known that NF-κB can be activated by DNA damage due to various agents, and NF-κB can display either apoptotic or anti-apoptotic effects, depending on the type of this DNA damage [48]. Drug-triggered nephrotoxicity studies indicate that a substantial portion of circulating IL-6 and TNF-α levels are derived from non-immune cells, probably the renal epithelial cells themselves, after CP administration. The production of TNF-α and IL-6 after CP administration is highly dependent upon NF-κB activation [5].

In our study, WT decreased TNF-α, IL-6 and NK-κB levels, demonstrating a protective effect on nephrotoxicity induced by CP. It has been well established that tea has anti-tumoral and anti-mutagenic effects. However, most of the studies in the literature addressing these effects are related to green tea. The number of studies on white tea is very limited. Both types of tea increase antioxidant enzyme capacity of different organs and protect against various diseases [49–51]. NF-κB, with a key role in the pathophysiology of clinically important diseases, stimulates the synthesis of proinflammatory cytokines [52]. This study shows that white tea inhibits inflammation associated with NF-κB, and the inhibition of NF-κB synthesis leads to the inhibition of TNF-α and IL-6 synthesis. In addition, white tea given before the development of CP toxicity is protective against CP-induced inflammation by inhibiting the activation of NF-κB. CP-induced kidney injury results in tubular dilation, obscured glomeruli, edema and focal areas of inflammation in the interstitium [53–55]. Ozyurt et al. [54] described severe vacuolation of the renal proximal tubular epithelium as well as cellular swelling and shedding. Iseri et al. [55] showed severe glomerular congestion and degeneration, dilatation in the Bowman's space, and degeneration in tubular cells.

In light of these studies, we set histopathological kidney evaluation criteria, and planned this study accordingly. In the histopathologic examination, the kidney sections of each group were examined in terms of tubular necrosis/atrophy, tubular vacuolar changes, glomerular damage, vascular congestion/ thrombosis, and interstitial inflammation, paying attention to their severity and distributions. Tubular necrosis, vascular congestion, and inflammation were observed in the CP group. The CP + WT group displayed a statistically significant improvement in terms of all parameters related to the damage when compared with the CP group.

Apoptosis, also called programmed cell death, is one of the most important events in the development of the organism and the maintenance of tissue homeostasis. The proteins that can activate apoptotic pathways are being targeted by the new therapies aimed at the destruction of tumor cells. CP too causes cell death via apoptosis similar to other drugs, and irregularities in these apoptotic pathways lead to CP resistance [56,57]. Hanigan and Devarajan [33] developed CP-induced nephrotoxicity in mice and observed apoptotic epithelial cells particularly in the distal tubules and collecting ducts. In addition, they claimed that CP administration caused mitochondrial dysfunction in renal epithelial cells, affecting the electron transport chain of the cell, and causing adenosine triphosphate (ATP) loss in the cell. With increasing doses of CP, accelerated ATP loss causes rapid metabolic collapse in the cell, leading to cell death.

Caspase activation is an indicator of cell damage in diseases. It was reported in the literature that caspase-3 activation was observed in CP-induced renal damage. Also caspase-3 activation due to CP was claimed to be mediated by a mechanism independent of mitochondrial dysfunction [58]. In our study, caspase 3 activation was investigated in kidney tissue. Numbers of normal tubular epithelial cells increased in the control group, whereas they decereased considerably as a result of CP-mediated apoptosis in parallel with caspase-3 activation. On the contrary, Caspase-3 levels decreased in the WT + CP group. The effect of WT on the levels of caspase 3 in kidney damage has not been studied in vivo models so far. However, there has been a report in which EGCG, one of the major catechin components of WT, was shown to decrease caspase 3 activity damaged rat kidneys. We think that white tea inhibits apoptosis, protecting the kidney cells against damage [59].

This study has a number of limitations. One of which is gender-specific effect/response. Although several studies showed the protective effect of estradiol on cisplatin-induced nephrotoxicity [30,60] in females, some studies reported no differences between genders [61]. In addition, while measuring the levels of inflammatory molecules in (TNF-α, IL-6 and NF-kB) in serum, their corressponding mRNA and/or protein levels in the kidney could be done for confirmation.

In conclusion, this study demonstrated that WT protects against CP induced kidney damage. The protective effect of white tea on CP nephrotoxicity may be attributed primarily to its anti-inflammatory and anti-apoptotic properties.

Conflict of interest

The authors declared no conflicts of interest.

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References

[1] P.E. Kintzel, Anticancer drug-induced kidney disorders: incidence, prevention and management, Drug Saf. 24 (2001) 19–38.
[2] J. Fillastre, M. Godin, et al., Drug-induced nephropathies, in: A.M. Davison, J.S. Cameron, J.P. Grünfeld (Eds.), Oxford Textbook of Clinical Nephrology, Oxford University Press, New York, 1998, pp. 2645–2657.
[3] A.M. Florea, D. Büsselberg, Anti-cancer drugs interfere with intracellular calcium Signaling, Neuro.Toxicol. 30 (2009) 803–810.
[4] B. Gultekin, A. Canbilen, Analyzing the effects of zinc on histopathological changes in rat testis caused by cisplatin toxicity, Acad. J. Sci. Res. 6 (2018) 008–017.
[5] R.P. Miller, R.K. Tadagavadi, G. Ramesh, W.B. Reeves, Mechanisms of cisplatin nephrotoxicity, Toxins (Basel) 2 (2010) 2490–2518.
[6] Z.H. Siddik, Cisplatin: mode of cytotoxic action and molecular basis ofresistance, Oncogene 22 (2003) 7265–7279.
[7] L. Arany, R.L. Safirstein, Cisplatin nephrotoxicity, Semin. Nephrol. 23 (2003) 460–464.
[8] N. Pabla, Z. Dong, Cisplatin nephrotoxicity: mechanisms and renoprotective strategies, Kidney Int. 73 (2008) 994–1007.
[9] W. Yan, Y. Xu, Yuan Y, L. Tian, Q. Wang, Y. Xie, X. Shao, M. Zhang, Z. Ni, S. Mou, A possible mechanism of cisplatin-induced tumor necrosis factor (TNF)-α production in murine macrophages, Pharmacol. Pharm. 4 (2013) 146–151.
[10] R.W. Schrier, Cancer therapy and renal injury, J. Clin. Invest. 110 (2002) 743–745.
[11] G.J. Dugbartey, L.J. Peppone, I.A. de Graaf, An integrative view of cisplatininduced renal and cardiac toxicities: molecular mechanisms, current treatment challenges and potential protective measures, Toxicology 371 (2016) 58–66.
[12] B. Zhang, G. Ramesh, C.C. Norbury, W.B. Reeves, Cisplatin-induced nephrotoxicity is mediated by tumor necrosis factor-α produced by renal parenchymal cells, Kidney Int. 72 (2007) 37–44.
[13] G. Ramesh, W.B. Reeves, TNF-a mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity, J. Clin. Invest. 110 (2002) 835–842.

[14] S. Akira, T. Taga, T. Kishimoto, Interleukin-6 in biology and medicine, Adv. Immunol. 54 (1993) 1–78.
[15] R.G. Baker, M.S. Hayden, S. Ghosh, NF-κB, inflammation and metabolic disease, Cell Metab. 13 (2011) 11–22.
[16] A.B. Sanz, M.D. Sanchez-Niño, A.M. Ramos, J.A. Moreno, B. Santamaria, M. Ruiz Ortega, J. Egido, A. Ortiz, NF-kappa B in renal inflammation, J. Am. Soc. Nephrol. 21 (2010) 1254–1262.
[17] C.S. Yang, J.M. Landau, Effects of tea consumption on nutrition and health, J. Nutr. 130 (2000) 2409–2412.
[18] F. Celik, Tea (Camellia sinensis); composition, the preventive effects on health and Consumption: review, Turkiye Klinikleri, J. Med. Sci. 26 (2006) 642–648.
[19] C. Espinosa, D. González-Silvera, F. P.érez-Llamas, J.A. López-Jiménez, S. Zamor, Effect of long term intake of white tea on acute oxidative stress in rats, Nutr. Hosp. 32 (2015) 749–756.
[20] T. Dias, G. Tomás, N. Teixeira, M. Alves, P. Oliveira, B. Silva, White tea (Camellia sinensis (L.)): antioxidant properties and beneficial health effects, Int. J. Food Sci. Nutr. Diet. 2 (2013) 19–26.
[21] G.C. Tenore, P. Stiuso, P. Campiglia, E. Novellino, In vitro hypoglycaemic and hypolipidemic potential of white tea polyphenols, Food Chem. 141 (3) (2013) 2379–2384.
[22] O. Ciftci, A. Cetin, M. Aydin, K. Kaya, F. Oguz, Fish oil, contained in eicosapentaenoic acid and docosahexaenoic acid, attenuates testicular and spermatological damage induced by cisplatin in rats, Andrologia 46 (2014) 1161–1168.
[23] S. Saral, E. Ozcelik, A. Cetin, O. Saral, N. Basak, M. Aydın, O. Ciftci, Protective role of diospyros lotus on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats, Andrologia 48 (2016) 308–317.
[24] J.F. Fangueiro, A. Parra, A.M. Silva, M.A. Egea, E.B. Souto, M.L. Garcia, A.C. Calpena, Validation of a high performance liquid chromatography method for the stabilization of epigallocatechin gallate, Int. J. Pharm. 475 (2014) 181–190, https://doi.org/10.1016/j.ijpharm.2014.08.053.
[25] B. Kacar, Chemical Analysis of Tea and Tea Soils, 1st edt., CAYKUR, Ankara, 1991.
[26] A.R. Nunes, M.G. Alves, G.D. Tomás, V.R. Conde, A.C. Cristóvão, P.I. Moreira, P.F. Oliveira, B.M. Silva, Daily consumption of white tea (Camellia sinensis (L.)) improves the cerebral cortex metabolic and oxidative profile in prediabetic wistar rats, Br. J. Nutr. 113 (2015) 832–842.
[27] M.J. Sung, D.H. Kim, Y.J. Jung, K.P. Kang, A.S. Lee, S. Lee, W. Kim, M. Davaatseren, J.T. Hwang, H.J. Kim, M.S. Kim, D.Y. Kwon, S.K. Park, Genistein protects the kidney from cisplatin-induced injury, Kidney Int. 74 (2008) 1538–1547.
[28] G. Akca, H. Eren, L. Tumkaya, T. Mercantepe, M.O. Horsanali, E. Deveci, E. Dil, A. Yilmaz, The protective effect of astaxanthin against cisplatin-induced nephrotoxicity in rats, Biomed. Pharmacother. 100 (2018) 575–582.
[29] V. Bunel, Y. Tournay, T. Baudoux, E. De Prez, M. Marchand, Z. Mekinda, R. Maréchal, T. Roumeguère, M.H. Antoine, J.L. Nortie, Early detection of acute cisplatin nephrotoxicity: interest of urinary monitoring of proximal tubular biomarkers, Clin. Kidney J. 10 (639) (2017) 647.
[30] A.M. Florea, D. Busselberg, Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects, Cancers (Basel) 3 (2011) 1351–1371.
[31] S.T. Ahmad, S. Sultana, Tannic acid mitigates cisplatin-induced nephrotoxicity in mice, Hum. Exp. Toxicol. 2 (2012) 145–156.
[32] A. Atessahin, A.O. Ceribasi, A. Yuce, O. Bulmus, G. Cikim, Role of ellagic acid against cisplatin induced nephrotoxicity and oxidative stress in rats, Basic Clin. Pharmacol. Toxicol. 100 (2007) 121–126.
[33] M.H. Hanigan, P. Devarajan, Cisplatin nephrotoxicity: molecular mechanisms, Cancer Ther. 1 (2003) 47–61.
[34] K. Sueishi, K. Mishima, K. Makino, Y. Itoh, K. Tsuruya, H. Hirakata, Protection by a radical scavenger edaravone against cisplatin-induced nephrotoxicity in rats, Eur. J. Pharmacol. 451 (2002) 203–208.
[35] T.G. Ahn, H.K. Kim, S.W. Park, S.A. Kim, B.R. Lee, S.J. Han, Protective effects of green tea polyphenol against cisplatin-induced nephrotoxicity in rats, Obstet. Gynecol. Sci. 57 (2014) 464–470.
[36] G.M. Anderson, M.T. Nakada, M. Dewitte, Tumor necrosis factoralpha in the pathogenesis and treatment of cancer, Curr. Opin. Pharmacol. 4 (2004) 314–320.
[37] A. Sklavounou, E. Crysomali, A. Scorilas, TNF-alpha expression and apoptosis regulating proteins in oral lichen planus, J. Oral Pathol. Med. 29 (2000) 5–370.
[38] K.J. Kelly, S.M. Meehan, R.B. Colvin, W.W. Williams, J.V. Bonventre, Protection from toxicant mediated renal injury in the rat with anti-CD54 antibody, Kidney Int. 56 (922) (1999) 931.
[39] J. Deng, Y. Kohda, H. Chiao, Y. Wang, X. Hu, S. Hewitt, T. Miyaja, P. McLeroy, B. Nibhanupudy, S. Li, R. Star, Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury, Kidney Int. 60 (2001) 2118–2128.
[40] I. Bekalp, B.A. Mamur, D.D. Yıldırım, L. Tamer, T. Çolak, N. Aras, Association of the polymorphisms and plasma level of interleuken-6 and interleuken-18 genes with colorectal cancer, Mersin Univ. Saglık Bilim. Derg. 7 (2014) 35–46.
[41] M.I. Yousef, H.M. Hussien, Cisplatin-induced renal toxicity via tumor necrosis factor-α, interleukin 6, tumor suppressor P53, DNA damage, xanthine oxidase, histological changes, oxidative stress and nitric oxide in rats: protective effect of ginseng, Food Chem. Toxicol 78 (2015) 17–25.
[42] P. Kumar, K. Sulakhiya, C.C. Barua, N. Mundhe, TNF-α, IL-6 and IL-10 expressions, Responsible for disparity in action of curcumin against cisplatin-induced nephrotoxicity in rats, Mol. Cell. Biochem. 431 (2017) 113–122.
[43] T. Lawrence, The nuclear factor NF-kappaB pathway in inflammation, Cold Spring Harb. Perspect. Biol. 1 (2009) a001651.
[44] A. Siomek, NF-kB signaling pathway and free radical impact, Acta Biochim. Pol. 59 (2012) 323–331.
[45] A. Kumar, Y. Takada, A.M. Boriek, B.B. Aggarwal, Nuclear factor-kappaB: its role in health and disease, J. Mol. Med. 82 (2004) 434–448.
[46] M.M. Elseweidy, M.S. Zaghloul, N.N. Younis, 10-DHGD ameliorates cisplatin-induced nephrotoxicity in rats, Biomed. Pharmacother. 83 (2016) 241–246.
[47] H.D. Francescato, T.M. Coimbra, R.S. Costa, M. de, L. Bianchi, Protective effect of quercetin on the evolution of cisplatin-induced acute tubular necrosis, Kidney Blood Press. Res. 27 (2004) 148–158.
[48] E. Strozyk, B. Pöppelmann, T. Schwarz, D. Kulms, Differential effects of NF kappa B on apoptosis induced by DNA-damaging agents: the type of DNA damage determines the final outcome, Oncogene 25 (2006) 6239–6251.
[49] G. Santana-Rios, G.A. Orner, A. Amantana, C. Provost, S.Y. Wu, R.H. Dashwood, Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay, Mutat. Res. 495 (2001) 61–74.
[50] A.E. Koutelidakis, K. Argiri, M. Serafini, C. Proestos, M. Komaitis, M. Pecorari, M. Kapsokefalou, Green tea, White tea, and Pelargonium purpureum increase the antioxidant capacity of plasma and some organs in mice, Nutrition 4 (2009) 453–458.
[51] A.E. Koutelidakis, M. Serafini, M. Komaitis, M. Kapsokefalou, Oxidative activity of some iron compounds on co lon tissue homogenates from mice after administration of green tea, white tea and Pelargonium purpureum, Food Chem. 120 (2010) 895–901.
[52] A.S. Baldwin Jr, Series introduction: the transcription factor NF-kappaB and human disease, J. Clin. Invest. 107 (2001) 3–6.
[53] H. Parlakpinar, E. Sahna, M.K. Ozer, F. Ozugurlu, N. Vardi, A. Acet, Physiological and pharmacological concentrations of melatonin protect against cisplatin induced acute renal injury, J. Pineal Res. 33 (2002) 161–166.
[54] H. Ozyurt, Z. Yildirim, M. Kotuk, H.R. Yilmaz, M. Yağmurca, M. Iraz, S. Sogut, S. Gergerlioglu, Cisplatin-induced acute renal failure is ameliorated by erdosteine in a dose dependent manner, J. Appl. Toxicol. 24 (2004) 269–275.
[55] S. Iseri, F. Ercan, N. Gedik, M. Yuksel, I. Alican, Simvastatin attenuates cisplatin-induced kidney and liver damage in rats, Toxicology 230 (2007) 256264.
[56] V.B. Cetintas, A.S. Kucukaslan, B. Kosova, A. Tetik, N. Selvi, G. Cok, C. Gunduz, Z. Eroglu, Cisplatin resistance induced by decreased apoptotic activity in non small cell lung cancer cell lines, Cell Biol. Int. 36 (2011) 261–265.
[57] B. Koberle, M.T. Tomicic, S. Usanova, B. Kaina, Cisplatin resistance: preclinical findings and clinical implications, Biochim. Biophys. Acta 1806 (2010) 172–182.
[58] B.S. Cummings, R.G. Schnellmann, Cisplatin-induced renal cell apoptosis: caspase 3 dependent and independent pathways, J. Pharmacol. Exp. Ther. 302 (2002) 8–17.
[59] S. Thangapandiyan, S. Miltonprabu, Epigallocatechin gallate supplementation protects against renal injury induced by fluoride intoxication in rats: role of Nrf2/HO-1 signaling, Toxicol. Rep. 1 (2014) 12–30.
[60] M. Nematbakhsh, Z. Pezeshki, F.E. Jazi, B. Mazaheri, M. Moeini, T. Safari, F. Azarkish, F. Moslemi, M. Maleki, A. Rezaei, S. Saberi, A. Dehghani, M. Malek, A. Mansouri, M. Ghasemi, F. Zeinali, Z. Zamani, M. Navidi, S. Jilanchi, S. Shirdavani, F. Ashrafi, Cisplatin-induced nephrotoxicity; protective supplements and gender differences, Asian Pac. J. Cancer Prev. 18 (2017) 295–314.
[61] M. Ghasemi, M. Nematbakhsh, Z. Pezeshki, N. Soltani, M. Moeini, A. Talebi, Nephroprotective effect of estrogen and progesterone combination on cisplatin-induced nephrotoxicity in ovariectomized female rats, Indian J. Nephrol. 26 (2016) 167–175.