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The Effects of White Tea (*Camellia Sinensis*) and Infliximab Against Cisplatin- Induced Testicular Damage via Oxidative Stress and Apoptosis

Efectos del Té Blanco (*Camellia Sinensis*) e Infliximab Contra el Daño Testicular Inducido por Cisplatino a Través del Estrés Oxidativo y la Apoptosis

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SUMMARY: Cisplatin (Cis) is an important chemotherapeutic agent used in cancer treatment. Males exposed to Cis were reported to exhibit testicular toxicity. Cis-induced testicular toxicity is mediated by oxidative stress, inflammation, testosterone inhibition and apoptosis. Accordingly, this study was conducted to evaluate the potential protective roles of infliximab (IFX), which is an anti-TNF-a agent, and of white tea (*Camellia sinensis*), which is known to possess antioxidant, anti-apoptotic, and anti-inflammatory effects, against Cis-induced testicular toxicity in rats. Rats were randomly assigned into five groups as follows: control group, Cisplatin (7 mg/kg) treatment group, Cisplatin (7 mg/kg) + infliximab (7 mg/kg) treatment group, cisplatin + white tea (WT) treatment group, and Cisplatin+ WT+IFX combined treatment group. In the present study, Cis exposure reduced the sperm count. It also increased testicular oxidative stress as well as the levels of inflammatory and apoptotic markers. Histopathological assays supported the biochemical findings. Treatment with IFX and/or WT restored testicular histology, preserved spermatogenesis, suppressed oxidative stress and apoptosis, and significantly ameliorated Cis-induced damage. It was concluded that white tea and infliximab could potentially serve as therapeutic options for the protection of testicular tissue against the harmful effects of Cis.

KEY WORDS: *Camellia Sinensis*; Cisplatin; Infliximab; Testis; Rat; White Tea.

INTRODUCTION

Cancer is an important health problem that is frequently encountered worldwide. The advances in cancer treatment have enabled an increase in the number of cancer survivors (Moradi *et al*., 2021). Although treating cancer was the main concern of physicians in the past, treating cancer-therapy related complications has become a major aspect of the problem. Particularly among young cancer survivors, infertility related to cancer treatments constitutes a significant problem (Sawaya *et al*., 2022; Wieder-Huszla *et al*., 2023). Specifically in the case of male infertility, sensitivity to chemotherapy, radiotherapy and oxidative stress is quite high due to the rapid cell cycle, low antioxidant capacity and high rate of polyunsaturated fatty acids in the testicular tissue (Ekinci Akdemir *et al*., 2019). Chemotherapy is among the primary treatment modalities used in the treatment of cancer. The medications used in chemotherapy result in a decrease in sperm viability and motility, as well as an impairment of quality, leading to infertility through various mechanisms, the most notable of which are oxidative stress and apoptosis (Tharmalingam *et al*., 2020; Bostancıeri *et al*., 2022). Several studies have been conducted to examine the antioxidative agents that were considered likely to eliminate the oxidative effects of chemotherapeutic medications for the purpose of reversing these negative effects and hence achieving fertilization (Lirdi *et al*., 2008; Reddy *et al*., 2016; Erfani Majd *et al*., 2021; Ghanbari *et al*., 2022).

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Cisplatin (Cis) is a platinum-based chemotherapeutic with proven effectiveness that is frequently used in the treatment of various cancers (Dasari & Tchounwou, 2014). It is thought to have multiple effect mechanisms; however, the most important of these mechanisms is that cisplatin results in the inhibition of DNA transcription and replication, and eventually cell apoptosis, by generating DNA crosslinks and DNA double strand breaks (Kitayama *et al*., 2022). Moreover, cisplatin leads to oxidative stress-mediated cell death by causing an increase in reactive oxygen species (ROS) (Dasari *et al*., 2022). In addition to its effectiveness in cancer treatment, cisplatin is known to also induce toxicity in many healthy organs, probably due to the above-mentioned mechanisms(Mercantepe *et al*., 2019; Elsayed *et al*., 2022). Testicular tissue is among the organs where cisplatin induces toxicity (Aly and Eid, 2020; Tharmalingam *et al*., 2020; Park *et al*., 2022). Cisplatin is known to result in infertility by germ cell apoptosis, Interstitial cells dysfunction, and the inhibition of testosterone production (Mori Sequeiros García *et al*., 2012; El-shafaei *et al*., 2018; Altındag & Meydan, 2021). Although the specific molecular mechanism underlying the toxicity of cisplatin in testicular tissue is not clearly known, the mechanism that is most strongly emphasized involves oxidative stress, inflammation, and apoptosis (Jahan *et al*., 2018; Bostancıeri *et al*., 2022). Therefore, a variety of antioxidant agents have been studied in the literature in order to reverse cisplatin-induced testicular damage (Aly & Eid, 2020; Wang *et al*., 2020; Altındagˇ & Meydan, 2021; Elsayed *et al*., 2022; Ijaz *et al*., 2022).

Cisplatin-induced oxidative stress causes an increase in the levels of proinflammatory cytokines that lead to the activation of the nuclear factor-κB (NF-κB) signaling pathway, which is the primary mediator of the inflammatory response (Habib *et al*., 2019; Makled & Said, 2021). Tumor necrosis factor-α (TNF-α) is one of the most important proinflammatory cytokines that induce apoptosis by triggering cell damage (Li *et al*., 2006; Moradi *et al*., 2021). It is also known to be involved in testicular pathophysiology (Li *et al*., 2006; Habib *et al*., 2019). High levels of TNF-α result in significant loss of spermatozoa function (Li *et al*., 2006). Antibodies developed against TNF-α were shown to improve sperm parameters and infertility in the literature (Habib *et al*., 2019). Infliximab (IFX) is a monoclonal antibody that targets TNF-a and is currently in use for the treatment for numerous inflammatory diseases (Amoroso *et al*., 2003; Aydin *et al*., 2014; Bahcecı *et al*., 2023).

Tea (*Camellia sinensis*) is a beverage that is widely consumed across the world. Studies in the literature that have been conducted on tea indicate an antioxidant and anti-inflammatory effect through the scavenging of ROS (Oliveira *et al*., 2015; Guvvala *et al*., 2017; Hassan *et al*., 2019). The antioxidant effect of tea is probably linked to its high polyphenol content (Figueiroa *et al*., 2009; Lopez *et al*., 2020). Tea has varieties such as white tea, green tea, black tea, and oolong tea depending on the time of harvest and the method of processing (Yu *et al*., 2010). Despite its lower consumption rate, white tea has the highest antioxidant capacity due to possessing the most abundant Epigallocatechin 3-gallate (EGCG) content (Oliveira *et al*., 2015). Although there are fewer studies in the literature on white tea (WT), it is known to have a high antioxidant capacity and an antihyperglycemic effect (Martins *et al*., 2014; Oliveira *et al*., 2015; Dias *et al*., 2016, 2017).

The negative effects of cancer treatment on fertility have directed researchers towards the discovery of agents targeting the preservation of fertility (Sabanegh & Ragheb, 2009). The presence of cisplatin-induced testicular damage is confirmed by the studies in the literature (Elsayed *et al*., 2022; Ijaz *et al*., 2022; Sawaya *et al*., 2022; Kuribayashi *et al*., 2023). However, to our knowledge, there are no studies in the literature that have investigated the use of white tea or infliximab for the reversal of cisplatin-induced testicular damage. Therefore, we aimed to demonstrate the effects of white tea, which is known to have been an effective antioxidant, and the TNF-a-antagonist infliximab on cisplatin-induced testicular damage. For this purpose, we treated cisplatin-treated rats with white tea and infliximab, both as single agents and in combination. We presented our results using histopathological and immunohistochemical methods, and biochemically by measuring malondialdehyde (MDA) and glutathione (GSH) levels. This is an exploratory study without hypothesis testing as there is no statistical null hypothesis corresponding to the alternative hypothesis that aims to prove our claims.

MATERIAL AND METHOD

In this study, 40 male Sprague-Dawley rats (3- to 4-month-old and weighing 312 ± 20 g) were used. The rats were obtained from Recep Tayyip Erdogˇan University Laboratory Animals Research Center. Ethics approval was granted by Recep Tayyip Erdogan University Laboratory Animals Local Ethics Committee on 26/12/2018 (2018/60). The rats were housed at a temperature of 22 ± 2 °C, humidity of 55 % ± 5, and a 12-hour light/darkness cycle. All rats had free access to standard pellet chow and water. The rats were randomized into five groups of eight rats each. The control group only received a single intraperitoneal (i.p.) dose of 0.09 % physiological serum. The Cisplatin (Cis) group received a single dose of 7 mg/kg cisplatin via the i.p. route. The Cis + White tea (WT) group received a single i.p. dose

of 7mg/kg cisplatin and white tea extract via oral gavage for four weeks, starting four weeks prior to the cisplatin treatment. The Cis+Infliximab (IFX) group received a single dose of 7 mg/kg cisplatin 72 h after the i.p. administration of 7 mg/kg infliximab. The Cis+WT+IFX group was treated with white tea, cisplatin and infliximab using the doses and durations described for the other groups.

In the end of the experiment, the rats were sacrificed under high dose anesthesia. Immediately after anesthesia, blood samples collected by intracardiac intervention were centrifuged at 4000 RPM for 10 min at +4 ºC. The left and right testicles were removed. The right testicles were dissected in half and stored at -80 ºC with serum samples to be used in biochemical assays. The left testicles were dissected in half and placed in Bouine's solution for histopathological assays and in 10 % neutral formalin to be evaluated in immunohistochemical analyses.

Chemicals and Preparation White Tea (*Camellia sinensis*) Extract. Ketamine hydrochloride (Ketalar, 100 mg/kg, Pfizer Drugs Ltd. Sti., Istanbul, Turkey), xylazine hydrochloride (Rompun, 10 mg/kg, Bayer, USA), fentanyl citrate (Talinat, 0.5 mcg/10 ml, Vem Pharmaceutical Industry Inc. (Ankara, Turkey). Cisplatin (Faulding Pharmaceuticals Plc, Warwickshire, UK). Infliximab (Remicade 100 mg/vial, MERCK SHARP DOHME Ilaclari LTD. STI.). All chemicals used in laboratory experiments were provided by Sigma Chemical Co. and Merck (Germany).

Camellia sinensis was obtained from CAYKUR A.S. (Rize, Turkey). Specimens (1.5g/100 mL) were infused with distilled water not exceeding 100 °C for 3 min as per the producer's instructions. The resulting infusion was cooled to room temperature and filtered through filter paper to be stored in dark bottles. The phenolic content as analyzed with high performance liquid chromatography (HPLC; ISO 9002 standard) and the mineral composition of WT as determined by the Kacar B method of analysis are listed in Tables I and II (Saral *et al*., 2019).

Table I. *Camellia sinensis* HPLC analysis results (100mg dry weight).

Catechins	%
Gallic acid	2.89
EGC	0
C	0.96
EC	5.31
EGCG	74.61
ECG	16.23
Total Catechins (EGC+C+EC+EGCG+ECG)	100

EGC:Epigallocatechin, C:catechin, EC: epicatechin, EGCG: epigallocatechin-3 gallate, ECG:epicatechin-3-gallate

Table II. Mineral content analysis of white tea extract.

Copper (Cu)	0.078 ppm 0.000078 g/kg	0.01 %
Iron (Fe)	0.128 ppm 0.000128 g/kg	0.02 %
Zinc (Zn)	1.34ppm 0.00134 g/kg	0.01 %
Sodium (Na)	4.18 ppm 0.00418 g/kg	0.31 %
Potassium (K)	1250 ppm 1.25 g/kg	92.90 %
Calcium (Ca)	14.51 ppm 0.01451 g/kg	1.08 %
Manganese (Mn)	3.11 ppm 0.00311 g/kg	0.21 %
Magnesium (Mg)	69.3 ppm 0.0693 g/kg	5.2 %
Aluminum (Al)	1.8 ppm 0.0018 g/kg	0.13 %
Total	1345,066 ppm 1.345066 g/kg	

Biochemical Analysis

Tissue homogenization. A preparation of 20 mM 1L sodium phosphate + 140 mM potassium chloride was made (pH 7.4). Then, 1 ml of homogenization solution was added onto 100 mg tissue, the testicular tissue was homogenized using a homogenizer (QIAGEN Tissue Lyser II), and centrifuged at 800 g for 10 min at 4 ºC. GSH and TBARS assays were performed with the obtained supernatant.

Malondialdehyde (MDA) Analysis Procedure (TBARS Assay). TBARS assay was performed according to the study by Ohkawa *et al*. (1979). A mixture of 200 µL tissue supernatant; 50 µL of 8.1 % SDS (sodiumdodecylsulphate); 375 µL of 20 % acetic acid(v/v) pH 3.5; 375 µL of 0.8 % thiobarbituricacid (TBA) was prepared. The mixture was vortexed, and the reaction was left to incubate in a boiling water bath for 1 h. After incubation, it was cooled in ice water for 5 min and centrifuged at 750 g for 10 min. The resulting pink color was measured with a spectrophotometer at 532 nm. The results were calculated in nmol/mg prt.

Glutathione (GSH) Analysis Procedure. Ellman's reagent was used to determine -SH groups (Sedlak & Lindsay, 1968). 250 µL supernatant was added with 1000 µL 3M Na2HPO4 and 250 µL DTNB (DNTB, 5,5'-dithiobis(2-nitrobenzoic acid) (4mg DTNB prepared in 10 mL 1 % sodium citrate solution), vortexed, and absorbance at 412 was determined. The results were determined using a predetermined 1000 µM- 62,5 µM reduced glutathione standard curve and presented in nmol/mg prt.

Histopathological Analyses. Rat testicular tissue specimens were fixed in Bouin's fixative (SigmaAldrich, St. Louis, MO, USA) for 36 hours. After fixation, dehydration was performed with ascending series of ethanol (30 %, 50 %, 70 %, 80 %, 90 %, 96 %, 100 % (two times) (Merck, Darmstadt, Germany). Then, the specimens were passed through two series of xylol (Merck, Darmstadt, Germany), mordanted, subjected to soft and hard paraffin (Merck, Darmstadt, Germany) inclusion in order, and lastly, blocked in hard paraffin. Preparations obtained by cutting sections of 3-5 μm with a microtome (Leica, RM2125RT, Germany) were stained with hematoxylin (Harris hematoxylin, Merck, Germany) and Eosin (H&E) (Eosin G, Merck, Germany). The preparations examined under a light microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan) were photographed using an Olympus DP71 (Olympus Corporation, Tokyo, Japan) camera.

Immunohistochemical (IHC) Analysis. Apoptotic cells were determined using TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) Assay Kit- HRP-DAB (ab206386, Abcam Inc., Cambridge, UK), the breakdown of reactive oxygen species (ROS) in the DNA was determined using 8-OHdG (8-hydroxy-2'-deoxyguanosine) (8-OHdG, ab62623, Cambridge, UK), and the activity of pro-inflammatory cytokines was determined using (Nuclear Factor kB/P65) NF-kB/p65 (1:100, ab16502, Abcam, UK) primary antibodies. Goat Anti-Rabbit IgG H&L (HRP) (ab205718) was used as the secondary antibody. Sections of 1-3 μm cut from paraffin blocks of testicular tissue using a microtome (Leica RM2125RT, Germany) were transferred onto positively charged slides (Patolab Biomedikal, Turkey). Following deparaffinization, testicular tissue sections were subjected to antigen retrieval according to the instructions for the primary antibodies. In this context, the sections were kept in 3 % H_2O_2 for 12 min, washed with phosphate buffer (Ph:7.4, Sigma-Aldrich, Germany), and kept in secondary blocking solution for 15 min. The sections were then incubated in primary and secondary antibodies for one hour each. After the diaminobenzidine (DAB) procedure, they were counterstained with Harris' hematoxylin (Merck, Darmstadt, Germany).

Semi-quantitative analysis. The histopathological analysis of H&E-stained testicular sections was performed by two histologists blinded to the experimental groups by calculating Johnsen scores44. In accordance with studies dealing with the toxicity of antineoplastic agents on testicular tissue, spermatogenetic cell count, edematous areas and vascular congestion findings were evaluated. Testicular Histopathological Damage Score (THDS) was calculated as shown in Table III. The histologists scored 20 different randomly selected regions per preparation.

Table III. Testis Histopathological Damage Score (THDS).

Score	Findings
Johnsen Score Rate (Control Group/Treatment Group)	
0	<5 %
1	<25 %
2	<50 %
3	≥50 %
Edematous Area	
0	<5 %
1	<25 %
2	<50 %
3	≥50 %
Vascular Congestion	
0	<5 %
1	<25 %
2	<50 %
3	≥50 %

The semi-quantitative analyses of TUNEL-, 8-OHdG-, NF-κB/p65- positive cells were evaluated as shown in Table IV (Gundersen, 1986; Mercantepe *et al*., 2016) (under x40 objectives). The histologists scored 20 different randomly selected regions per preparation.

Table IV. Immunohistochemical Positivity Score.

Score	Results
0	<5 %
1	<25 %
2	<50 %
3	≥50 %

Statistical Analysis. All data obtained from the analyses in this study were computed using the SPSS 18.0 (IBM, Armonk, NJ, USA) statistics software. Data from semiquantitative analyses were calculated as median±standard deviation in consideration of maximum and minimum values. After the differences between the groups were analyzed using the non-parametric Kruskall Wallis test followed by the Tamhane T2 test, the numeric data (biochemical analyses) from the groups were subjected to analyses ($p<0.05$ was accepted as significant). The obtained parametric data were calculated as arithmetic mean±standard deviation. The differences between the groups were analyzed using ANOVA followed by the Tukey HSD test ($p<0.05$ was accepted as significant).

RESULTS

Biochemical Analysis

Malondialdehyde (MDA) levels. We determined higher MDA levels in the Cis group compared to the control group

(Table V, p=0.01). In contrast, we observed significantly reduced testicular MDA levels in the Cis+WT and Cis+IFX groups compared to the Cis group (Table V, p=0.01). Similarly, we determined significantly reduced testicular MDA levels in the Cis+WT+IFX combination group compared to the Cis group (Table V, p=0.01).

Glutathion (GSH) Levels. We determined reduced GSH levels in the Cis group compared to the control group (Table V, p=0.01). In contrast, GSH levels were significantly higher in the Cis+WT and Cis+IFX treatment groups compared to the Cis group (Table V, p=0.01). Similarly, we determined significantly higher GSH levels in the Cis+WT+IFX group compared to the Cis group (Table V, p=0.01).

Histopathological Analysis. On histological evaluation of the control group, we observed seminiferous tubules packed with Sustentacular cells, spermatogonia, primary spermatocytes and spermatids of normal structure. In addition, there were numerous spermatozoa of normal structure in the seminiferous tubules and Interstitial cells in the interstitial spaces (Fig. 1a-b, Tables VI-VII, Johnsen Score: 9.36±0.64, TDS score: 1(1-2)). In contrast, in the Cis treatment group, we determined a decrease in spermatogenetic cells, most notably in primary spermatocytes and spermatids in the seminiferous tubule epithelium. In addition, there was extensive necrosis accompanied by edematous areas in spermatogenetic cells of the germinal epithelium. We observed vascular congestion and atypical Interstitial cells in the interstitial spaces (Fig. 1c-d, Tables VI-VII, Johnsen Score: 4.16±1.07, TDS score: 6(6-7)). In the Cis+WT group, we determined an increase in the number of spermatogenetic cells in the seminiferous tubules in comparison to the Cis treatment group. In addition, we observed a decrease in edematous areas and in the vascular congestions in the interstitial spaces (Fig. 1e-f, Table VI-VII, Johnsen Score: 7.76±0.78, TDS score: 2(1-2)). Similarly, we observed an increase in the number of spermatogenetic cells, most notably in primary spermatocytes and spermatids. In addition, we determined fewer necrotic germinal epithelial cells and a decrease in the vascular congestions in the interstitial spaces (Fig. 1g-h, Tables VI-VII, Johnsen Score: 8.08±0.71, TDS score: 2(1-2)). In the Cis+WT+IFX group, we observed reduced necrosis in epithelial cells in the seminiferous tubules compared to the Cis group. In addition, spermatogenetic cells of typical structure were common in the seminiferous tubules. We observed Interstitial cells of typical structure and reduced vascular congestion in the interstitial spaces (Fig. 1i-j, Tables VI-VII, Johnsen Score: 8.32±0.6, TDS score: 1(1-2)).

Immunohistochemical Analysis. TUNEL Analysis (The terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling)

In the Cis group, we observed an increase in the number of TUNEL-positive cells among spermatogenetic cells in the seminiferous tubules compared to the control group (Fig. 2a-b, Table VIII, p=0.001, TUNEL positivity score: 2(2-3)). In contrast, we determined a decrease in the number of TUNEL-positive spermatogenetic cells in the Cis+WT and Cis+IFX treatment groups compared to the Cis treatment group (Fig. 2c-d, Table VIII, p=0.001, TUNEL positivity score: 0(0-1), TUNEL positivity score: 0(0-0); respectively). Similarly, in the Cis+WT+IFX group, we determined a decrease in the number of cells showing TUNEL immunopositivity (Fig. 2e, Table VIII, p=0.001, TUNEL positivity score: 0(0-1)).

8-OHdG (8-hydroxy-2-deoxyguanosine) Analysis. In the Cis group, we observed an increase in 8-OHdG positivity in spermatogenetic cells in the seminiferous tubules compared to the control group (Fig. 3a-b, Table VIII, p=0.001, 8-OHdG positivity score: 2(2-3)). In contrast, we determined a decrease in spermatogenetic cells showing 8-OHdG immunopositivity in the Cis+WT, Cis+IFX and Cis+WT+IFX treatment groups compared to the Cis treatment group (Fig. 3c, Table 9, p=0.001, 8-OHdG positivity score: 0(0-1); Fig. 3d, Table VIII, p=0.001, 8-OHdG positivity score: 0(0-0); Fig. 3e, Table VIII, p=0.001, 8-OHdG positivity score: 0(0-0) respectively).

Table V. Biochemical Analysis Results (mean±standard deviation).

Group	MDA (nmol/mg tissue)	GSH (nmol/mg tissue)
Control	0.54±0.43	41.01±1.20
Cisplatin (Cis)	1.23±0.11[a]	27.90±1.70[a]
Cis+Infliximab (IFX)	0.83±0.64[a,b]	36.38±1.25[a,b]
Cis+White Tea (WT)	0.87±0.54[a,b]	35.82±1.49[a,b]
Cis+WT+IFX	0.76±0.28[a,b]	38.36±0.93[c,d]

[a] p=0.001 *versus* to Control Group,
[b] p=0.001 *versus* to Cis Group,
[c] p=0.017 *versus* to Control Group,
[d] p=0.023 *versus* to Control Group,
One-Way ANOVA-Tukey HSD

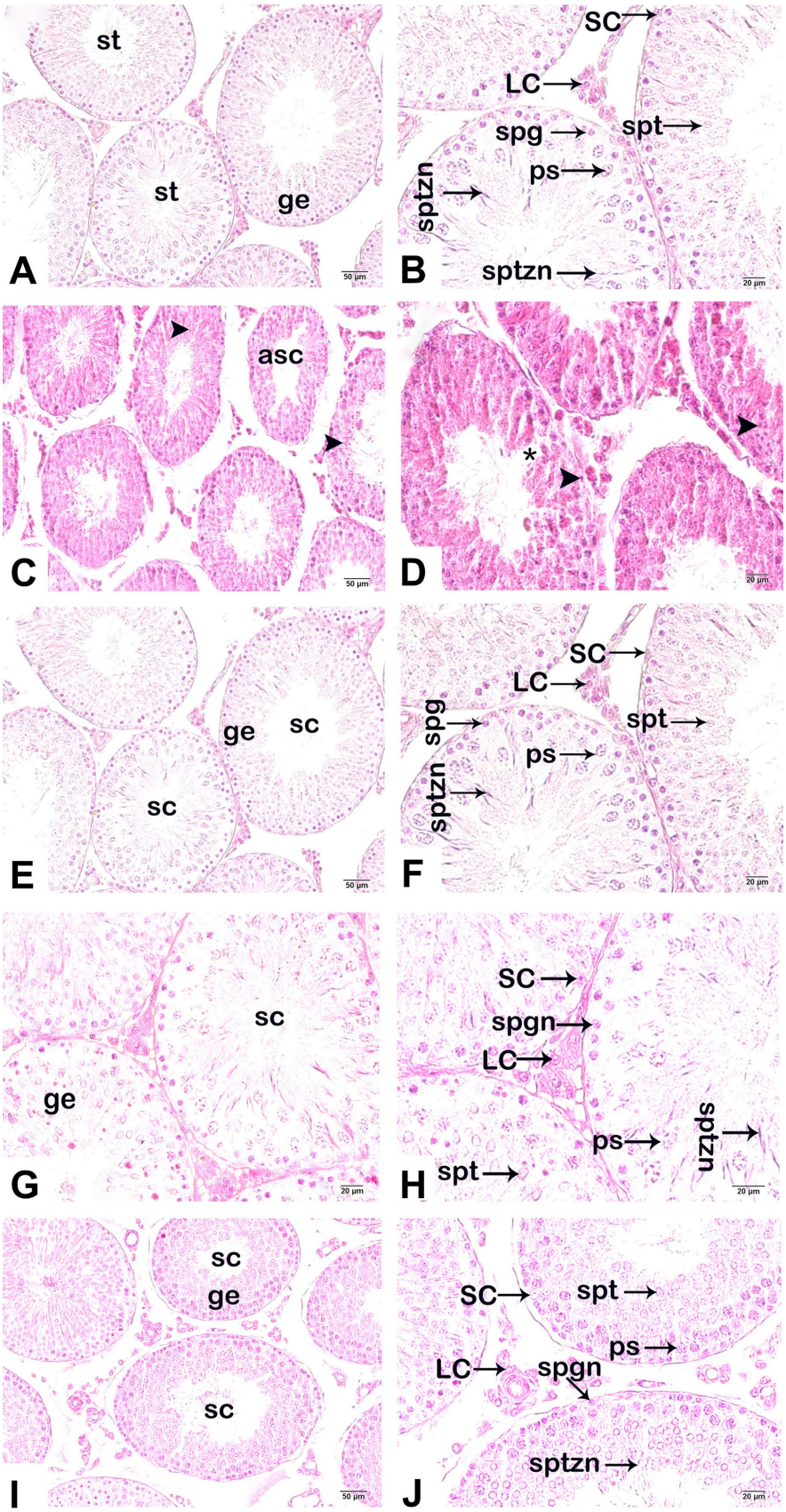

Fig. 1. Representative light microscopic picture of sections of testicular tissue stained with Harris Hematoxylin and Eosin G. Spermatogonium (spgn), Primary spermatocyte (ps), spermatid (spt), spermatozoon (sptzn), Sustentacular cells (SC), Interstitial cells (LC). A.(20×)-B(40×): Normal germinal epithelium (ge) containing normal spermatogonia (spgn), primary spermatocyte (ps), spermatid (spt), spermatozoon (sptzn) and Sustentacular cells (SC) in testicular tissue sections of the control group being watched. In addition, Interstitial cells with a typical structure are observed in the intertubular areas (THDS: 1(1-2)). C. (20×)-D(40×): We detected pycnotic nuclei (arrowhead) of spermatogenetic cells, primarily spermatozoa, and spermatids, in the seminiferous tubules of the cisplatin administration group. In addition, edematous areas (asterisk) and vascular congestions are observed due to losses in the germinal epithelium (THDS: 6(6-7)). E. (20×)-F(40×): It is observed that pycnotic cells and edematous areas in the germinal epithelial cells in the seminiferous tubules are decreased in the testicular tissue belonging to the infliximab administration group (THDS: 2(1-2)). G. (20×)-H(40×): It can be observed that the pycnotic nuclei of spermatogenetic cells, especially spermatozoa, and spermatids, decreased in testicular tissue sections belonging to the White tea application group. In addition, we observed a decrease in edematous areas and vascular congestions in the germinal epithelium (THDS: 2(1-2)). I. (20×)-J(40×): It is observed that pycnotic nuclei structures are decreased in spermatogenetic cells in seminiferous tubules. In addition, it is observed that the germinal epithelium in the seminiferous tubules has a typical structure (THDS: 1(1-2)).

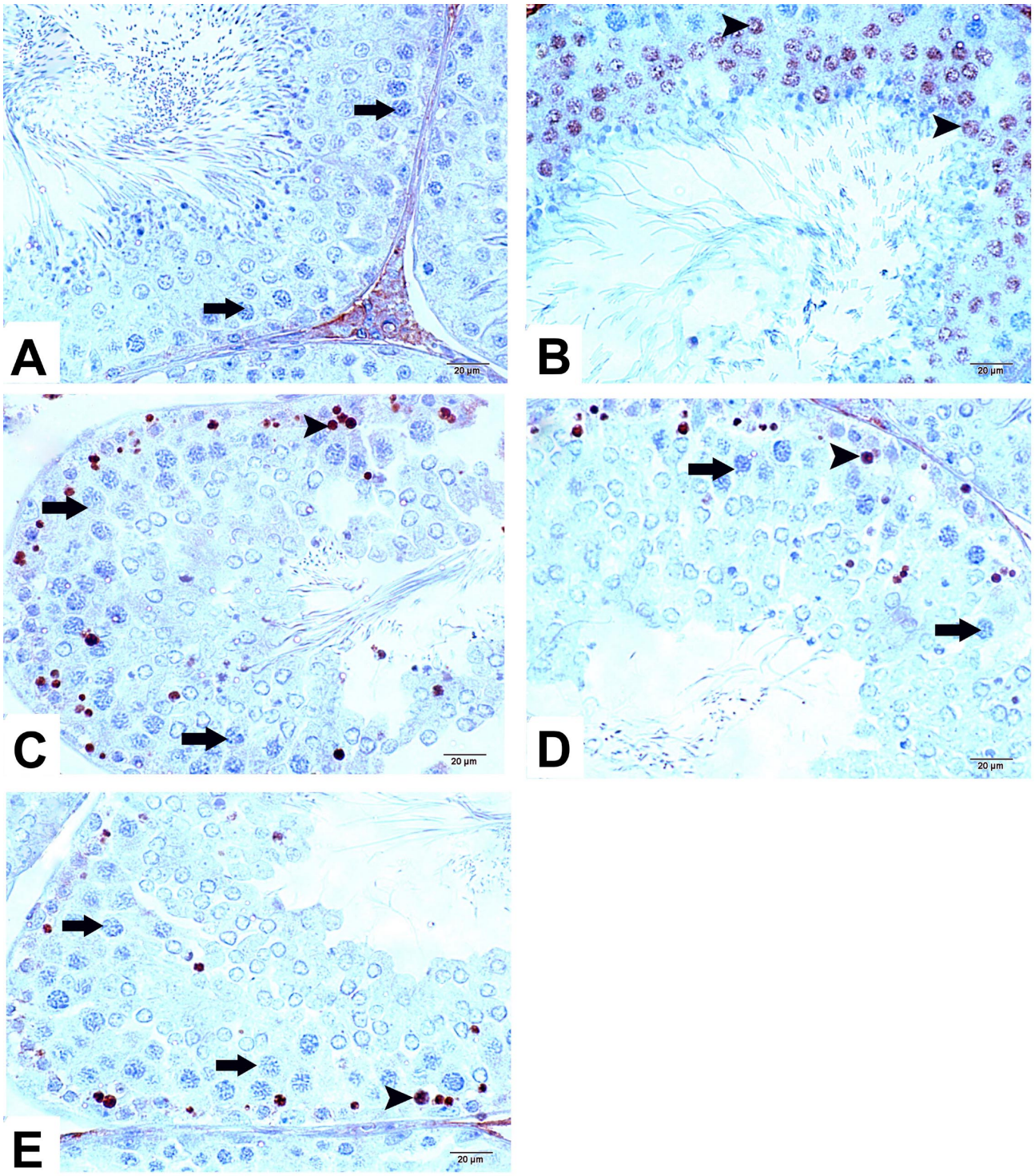

Fig. 2. Representative light microscopic picture of sections of testicular tissue. Apoptotic cells labeled with TUNEL methods. A. (40×) Control Group: It is observed that spermatogenic cells with normal structures in the germinal epithelium in the seminiferous tubules are immune-negative (TUNEL Positivity Score: 0(0-0)). B. (40×) Cis Group: Intense immune positivity is observed in apoptotic cells in the seminiferous tubules (arrowhead, TUNEL Positivity Score: 2(2-3)). C. (40×) Cis+ IFX Group: It is observed that the spermatogenic cells showing intense immuno-positiveness in the seminiferous tubules are decreased (TUNEL Positivity Score: 0(0-1)). D. (40×) Cis+WT Group: It is observed that cells with intense TUNEL positivity are decreased in the seminiferous tubules, especially in spermatids and spermatozoa (TUNEL Positivity Score: 0(0-0)). E. (40×) Cis+IFX+WT Group: It is observed that dense immune-positive spermatogenic cells decreased in the seminiferous tubules (TUNEL Positivity Score: 0(0-0)).

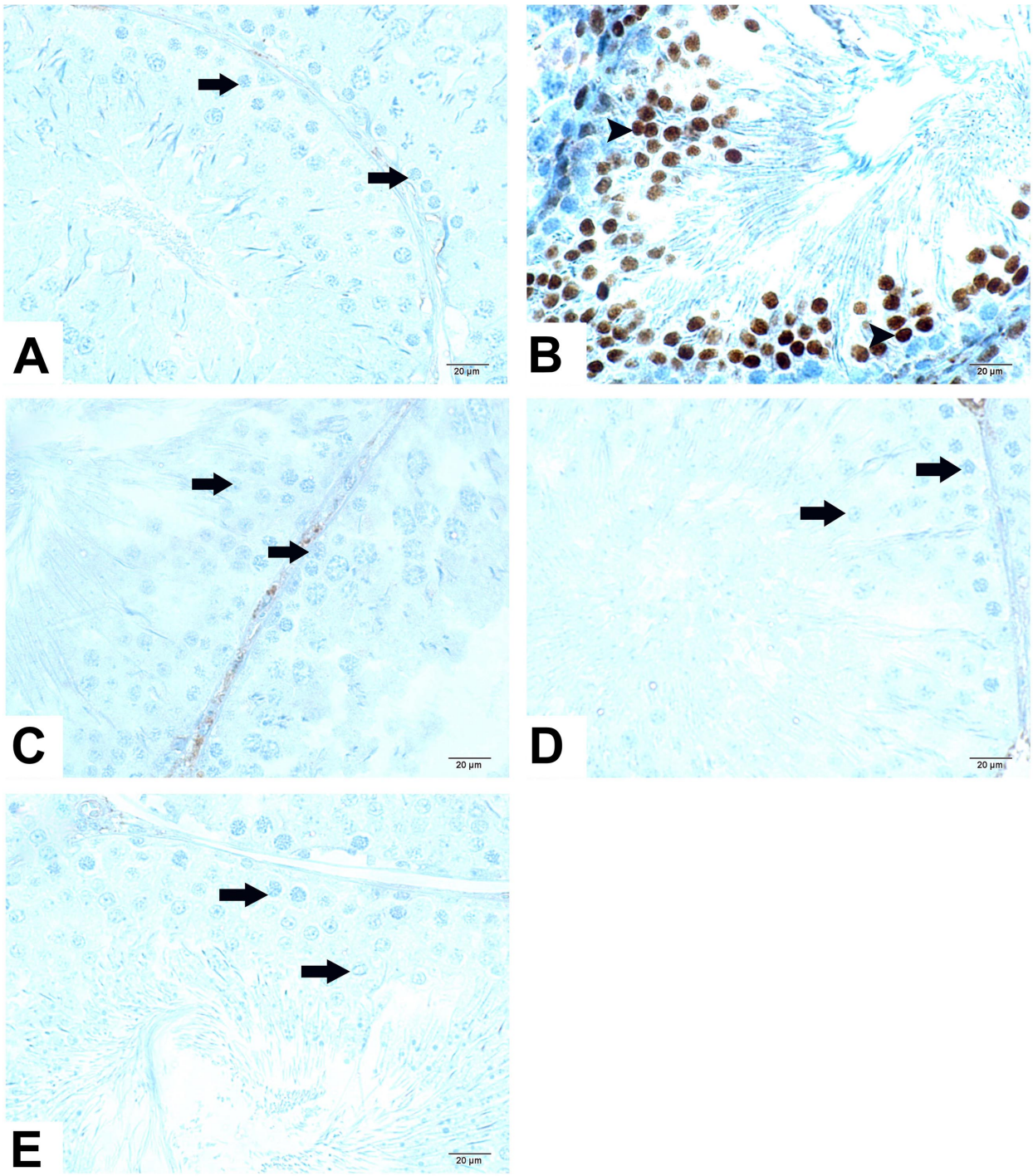

Fig. 3. Representative light microscopic picture of sections of testicular tissue incubated with 8-OHdG primary antibody. A. (40×) Control Group: It is observed that Sustentacular cells, Interstitial cells and spermatogenic cells with normal structures in the germinal epithelium in the seminiferous tubules are immune-negative (8-OHdG Positivity Score: 0(0-0)). B. (40×) Cis Group: Intense 8-OHdG positivity is observed in spermatogenetic cells in the seminiferous tubules, especially spermatozoon and spermatids (arrowhead), 8-OHdG Positivity Score: 2(2-2)). C. (40×) Cis+ IFX Group: It is observed that the germinal epithelial cells and Interstitial cells showing decreased intense 8-OHdG -positiveness in the seminiferous tubules are decreased (8-OHdG Positivity Score: 0(0-1)). D. (40×) Cis+WT Group: It is observed that intense 8-OHdG positive spermatogenetic cells decreased in the seminiferous tubules, especially in spermatids and spermatozoa (8-OHdG Positivity Score: 0(0-1)). E. (40×) Cisplatin+IFX+WT Group: It is observed that decreased dense immune-positive germinal epithelial and Interstitial cells in the seminiferous tubules (8-OHdG Positivity Score: 0(0-0)).

Table VI. Johnsen testis damage score results (median-25 %-75 % interquartile range).

Group	Johnsen Score Results	Johnsen Score Rate: Control/Treament Group
Control	9.36±0.64	9.6/9.6:1
Cisplatin (Cis)	4.16±1.07[a]	9.6/4.16:2.25:2
Cis+Infliximab (IFX)	7.76±0.78[a,b]	9.6/7.76:1.23:1
Cis+White Tea (WT)	8.08±0.71[a,b]	9.6/8.08:1.18:1
Cis+WT+IFX	8.32±0.63[a,b]	9.6/8.32:1.12:1

[a]p=0.001 *versus* to Control Group,
[b]p=0.001 *versus* to Cis Group,
[c]p=0.017 *versus* to Control Group,
[d]p=0.023 *versus* to Control Group,
One-Way ANOVA-Tukey HSD

Table VII. Testis Histopathological damage score (THDS, median-25 %-75 % interquartile range).

Group	Johnsen Score Rate	Edema	Vascular Congestion	THDS
Control	1	0(0-0)	0(0-0)	1(1-2)
Cisplatin (Cis)	2	2(2-2)[a]	2(2-2)[a]	6(6-7)[a]
Cis+Infliximab (IFX)	1	0(0-1)[b]	0(0-1)[b]	2(1-2)[b]
Cis+White Tea (WT)	1	0(0-0)[b]	0(0-1)[b]	2(1-2)[b]
Cis+WT+IFX	1	0(0-1)[b]	0(0-1)[b]	1(1-2)[b]

[a]p=0.001 *versus* to Control Group,
[b]p=0.001 *versus* to Cis Group,
One-Way ANOVA-Tukey HSD

Table VIII. Immunohistochemical positivity grading score (median-25 %-75 % interquartile range).

Group	Tunel	8-OHdG	NF-kB/p65
Control	0(0-0)	0(0-0)	0(0-0)
Cisplatin (Cis)	2(2-3)[a]	2(2-2)[a]	3(2-3)[a]
Cis+ Infliximab (IFX)	0(0-1)[b]	0(0-1)[b]	0(0-1)[b]
Cis+ White Tea (WT)	0(0-0)[b]	0(0-1)[b]	0(0-0)[b]
Cis+WT+IFX	0(0-0)[b]	0(0-0)[b]	0(0-0)[b]

[a]p=0.001 *versus* to Control Group,
[b]p=0.001 *versus* to Cis Group,
One-Way ANOVA-Tukey HSD

Nf-Kb/p65 Analysis. There was an increase in the number of spermatogenetic cells, most notably of primary spermatocytes and spermatids, showing NF-kb/p65-positivity in seminiferous tubules in the Cis treatment group compared to the control group (Fig. 4a-b, Table VIII, p=0.001, NF-kb/p65 positivity score: 2(2-3)). In contrast, we determined a decrease in spermatogenetic cells, most notably in primary spermatocytes and spermatids showing NF-kb/p65-positivity in the Cis+WT and Cis+IFX treatment groups compared to the Cis treatment group (Fig. 4c-d, Table VIII, p=0.001, NF-kb/p65: 0(0-1), NF-kb/p65 positivity score: 0(0-0); respectively). Similarly, we determined a decrease in the number of spermatogenetic cells showing NF-kb/p65 positivity in the Cis+WT+IFX group (Fig. 4e, Table VIII, p=0.001, NF-kb/p65 positivity score: 0(0-0)).

DISCUSSION

This study investigated the effects of white tea and/or infliximab on testicular damage induced by cisplatin using a rat model. In line with the literature, our results showed the development of cisplatin-induced damage in testicular tissues. However, the cisplatin-induced damage in testicular tissue recovered either partially or completely with the administration of white tea and infliximab, both as single agents and in combination. Although there is a multitude of studies showing the occurrence of cisplatin-induced testicular toxicity, the molecular mechanism of cisplatin-related testicular damage has not been completely clarified (Narayana *et al*., 2009; Aly & Eid, 2020; Matilionyte *et al*., 2022; Ismail *et al*., 2023). However, the most widely studied and the most strongly emphasized mechanism is associated with oxidative stress and increased apoptosis (Salem *et al*., 2012; Azab *et al*., 2020). Indeed, cisplatin increases ROS via the breaks it causes in the DNA (Zhang *et al*., 2022). The resulting ROS cause an increase in the production of proinflammatory cytokines (Tian *et al*., 2018). Elevated levels of proinflammatory cytokines, particularly of TNF-α, result in the activation of the nuclear factor-κB (NF-κB) signaling pathway, which leads to inflammation and apoptosis (Makled & Said, 2021). High levels of TNF-α also accelerate inflammation by increasing the production

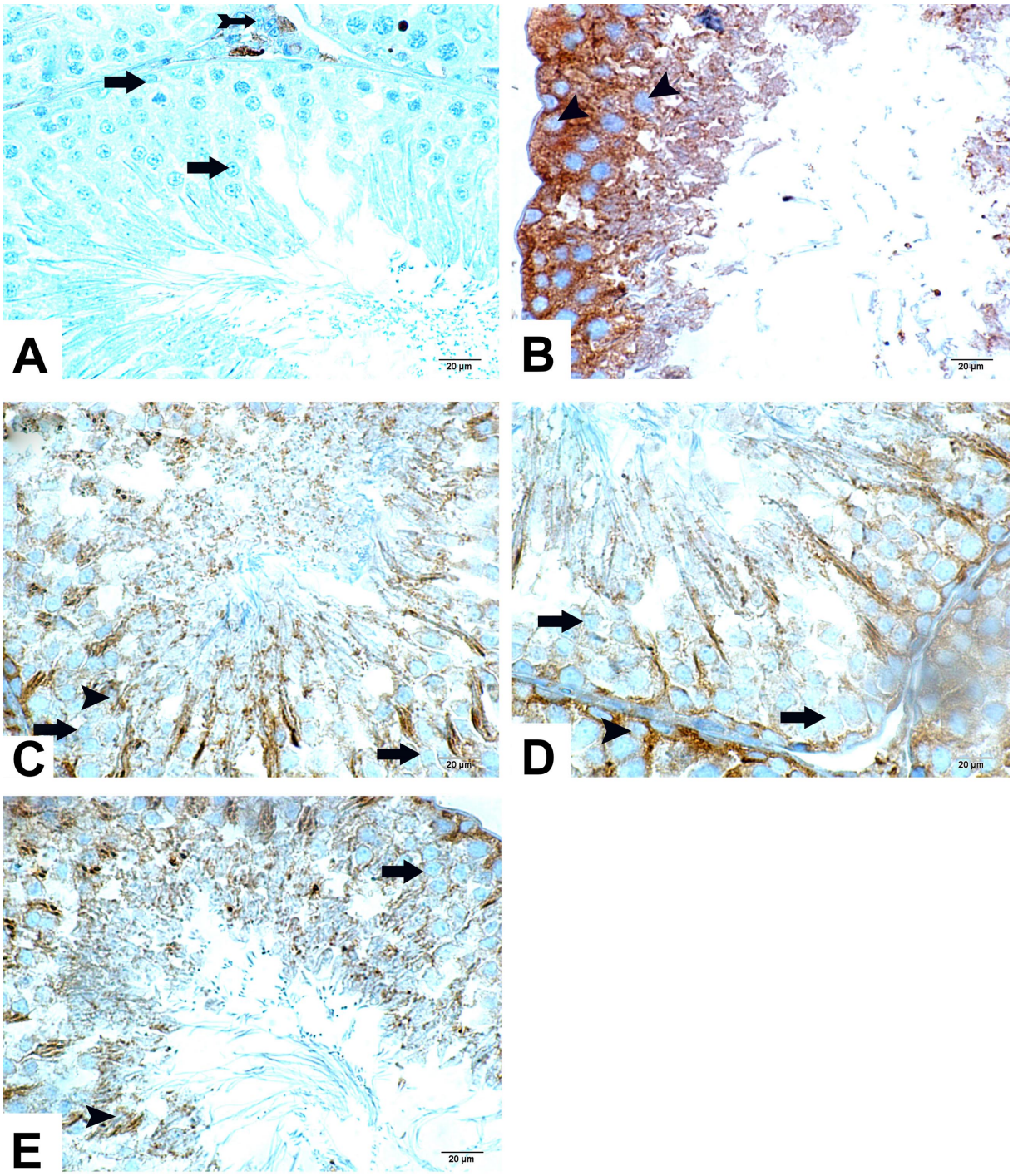

Fig. 4. Representative light microscopic picture of sections of testicular tissue incubated with NF-κB/p65 primary antibody. A. (40×) Control Group: It is observed that germinal epithelial and Interstitial cells with normal structures in the seminiferous tubules are NF-κB/p65-negative (NF-κB/p65 Positivity Score: 0(0-0)). B. (40×) Cis Group: Intense NF-κB/p65 positivity is observed in spermatogenetic cells in the seminiferous tubules, especially spermatozoon and spermatids (arrowhead), NF-κB/p65 Positivity Score: 3(2-3)). C. (40×) Cis+IFX Group: It is observed that the spermatogenetic cells in the seminiferous tubules show decreased intense NF-κB/p65-positiveness in the seminiferous tubules (NF-κB/p65 Positivity Score: 0(0-1)). D. (40×) Cis+WT Group: It is observed that intense NF-κB/p65 positive spermatogenetic cells decreased in the seminiferous tubules, especially in spermatids and spermatozoa (NF-κB/p65Positivity Score: 0(0-0)). E. (40×) Cis+IFX+WT Group: It is observed that decreased immune-positive spermatogenetic cells in the seminiferous tubules (NF-κB/p65 Positivity Score: 0(0-0)).

of prostaglandins (Habib *et al*., 2019). It was shown that high TNF-a levels could impair testicular function (Bhat *et al*., 1999). High levels of TNF-α have previously been implicated in testicular dysfunction in males with rheumatoid arthritis and infertility in females with endometriosis (Habib *et al*., 2019). The positive effects of treatments targeting TNF-a on fertilization have been demonstrated in clinical and experimental studies(Habib *et al*., 2019). Indeed, infliximab, which is a TNF-α-antagonist that was used in the present study, exhibited a protective effect against cisplatin-induced testicular toxicity. Infliximab restored the testicular architecture that was disrupted by cisplatin and improved the oxidant-antioxidant status in favor of antioxidants. It also increased the sperm count. To our knowledge, there are no studies in the literature investigating the effects of infliximab against cisplatin-induced testicular damage. However, there is a study by Habib *et al*. (2019) showing that infliximab treatment restores testicular function and architecture in cadmium-induced testicular toxicity. This was linked to the antioxidant effect of infliximab that reduces ROS by decreasing TNF-α levels. In our study, the administration of infliximab 72 hours prior to the cisplatin injection (since infliximab reaches a steady plasma concentration in 72 hours) (Cumhur Cüre *et al*., 2016), resulted in a significant decrease in the levels of MDA, which is a lipid peroxidation marker, while increasing the levels of GSH, which is considered one of the most potent antioxidants. At the same time, TNF-α-activated NF-κB positivity was found to be lower in rat testicular tissues treated with infliximab. NF-κB activation is the basic event marking the initiation of inflammation (Katturajan *et al*., 2021). This may indicate that infliximab also exerts an anti-inflammatory effect on testicular tissues. TNF-α causes cell death by activating the caspase cascade through TNFR1, which is one of its two receptors (Li *et al*., 2006). The reduced TUNEL positivity in rats treated with infliximab may indicate reduced necrosis or stem from a decrease in apoptosis.

Tea has been a widely consumed beverage and the subject of extensive discussion across the world. It has varieties such as white tea, green tea, black tea and oolong tea, and this diversity is caused by local differences, the time of harvest and differences in fermentation. The polyphenols found in tea were shown to be non-toxic and even have protective effects against ROS (Koonyosying *et al*., 2019). EGCG is a catechin found in tea that possesses the strongest antioxidant and anti-inflammatory properties (Pan *et al*., 2017; Hassan *et al*., 2019). And the highest EGCG content is found in white tea (Dias *et al*., 2016). Therefore, we preferred to use white tea in this study. Although green tea in particular has been widely researched in the literature, studies on white tea are relatively fewer. There are limited studies showing the antioxidant effects of white tea extract on prediabetic rat testicular tissues and Sustentacular cells culture media (Martins *et al*., 2014; Oliveira *et al*., 2015; Saral *et al*., 2019). Similarly, our study showed that white tea extract treatment reduced cisplatin-induced oxidative stress and restored the normal testicular histological structure. Moreover, there is an adequate amount of studies showing that EGCG exerts protective effects on testicular tissue in various conditions of oxidative stress (Yu *et al*., 2010; Guvvala *et al*., 2017; Hassan *et al*., 2019; Al-Maghrebi *et al*., 2020). This protective effect on testicular tissue was considered to be linked to the antioxidant quality of EGCG.

Our study is an exploratory pilot study that investigates the effects of white tea and infliximab on testicular damage induced by cisplatin. As we designed this study, we were particularly conscious of the fact that the agents selected as antioxidants should not reduce the effectiveness of the medications used in cancer treatment or exhibit tumor-protective properties. Our review of the literature showed both white tea and infliximab to be free of these effects. Tea had an antitumor effect due to its high EGCG content (Kuo & Lin, 2003). Although TNF-a is required for tumor apoptosis, its high levels entailed tumor cell invasion and metastasis (Perdigoto *et al*., 2022). Moreover, there were no case reports in the literature suggesting that infliximab exacerbates cancer; on the contrary, there were reports suggesting that it prevents oxaliplatin resistance in colon cancer patients (Huang *et al*., 2018). Nevertheless, this study should be interpreted in consideration of certain limitations. In the design of this study, we did not construct sham groups for white tea and infliximab. We were not able to measure the levels of testosterone, FSH, LH and androgenic steroids in this study. This study is an animal model that needs to be corroborated by clinical studies.

CONCLUSION

In summary, the present study confirmed that cisplatin induces testicular damage through oxidative stress and apoptosis, in line with previous studies. In addition, we showed white tea extract and the TNF-α-antagonist infliximab to exert protective effects against cisplatin-induced testicular damage by reducing oxidative stress and necrosis. The degrees of the protective effects of white tea and infliximab on the testicles were quite comparable. The combined administration of the two agents exerted the same protective effect and was not superior. Future animal and human studies are needed to elucidate this effect and the molecular mechanism that underlies it.

CAKIROGLU, S.; MERCANTEPE, T.; TUMKAYA, L.; UYDU, H. A.; TOPCU, A. & ATAK, M. Efectos del té blanco (*Camellia sinensis*) e infliximab contra el daño testicular inducido por cisplatino a través del estrés oxidativo y la apoptosis. *Int. J. Morphol., 41(5)*:1537-1549, 2023.

RESUMEN: El cisplatino (Cis) es un importante agente quimioterapéutico utilizado en el tratamiento del cáncer. Se informó que los hombres expuestos a Cis exhibieron toxicidad testicular. La toxicidad testicular inducida por Cis está mediada por el estrés oxidativo, la inflamación, la inhibición de la testosterona y la apoptosis. En consecuencia, este estudio se realizó para evaluar las posibles funciones protectoras de infliximab (IFX), un agente anti-TNF-α, y del té blanco (*Camellia sinensis*), conocido por sus propiedades antioxidantes, antiapoptóticas y anti-TNF-α -efectos inflamatorios, contra la toxicidad testicular inducida por Cis en ratas. Cinco grupos de ratas se asignaron al azar de la siguiente manera: grupo control, grupo de tratamiento con cisplatino (7 mg/kg), grupo de tratamiento con cisplatino (7 mg/kg) + infliximab (7 mg/kg), grupo de tratamiento con cisplatino + té blanco (WT), y grupo de tratamiento combinado Cisplatino+ WT+IFX. En el presente estudio, la exposición a Cis redujo el conteo de espermatozoides. También aumentó el estrés oxidativo testicular, así como los niveles de marcadores inflamatorios y apoptóticos. Los ensayos histopatológicos respaldaron los hallazgos bioquímicos. El tratamiento con IFX y/o WT restauró la histología testicular, preservó la espermatogénesis, suprimió el estrés oxidativo y la apoptosis, y mejoró significativamente el daño inducido por Cis. Se concluyó que el té blanco y el infliximab podrían potencialmente servir como opciones terapéuticas para la protección del tejido testicular contra los efectos nocivos de Cis.

PALABRAS CLAVE: *Camellia sinensis*; Cisplatino; Infliximab; Testículo; Rata; Té blanco.

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