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Assessment of Resveratrol's effects comparatively with zinc in experimental rat testicular damage induced by Cyclophosphamide

Siklofosfamid'in neden olduğu deneysel sıçan testis hasarında Resveratrol'ün etkilerinin çinko ile karşılaştırmalı olarak değerlendirilmesi

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ABSTRACT

Aim: To investigate protective effects of Resveratrol in combination or comparison with zinc in experimental testicular injury induced by Cyclophosphamide is studied for the first time in literature.

Materials and Methods: Rats (n=63) were randomly divided into 9 groups. After 21 days of drug administration biochemical and histological analysis were performed. Daily water consumption, body weights and weight of testes were measured. Johnsen's testicular scoring and sperm morphology were evaluated. Hematoxylin&Eosin, Periodic Acid-Schiff and Masson's Trichrome stainings and iNOS, eNOS and CD34 antibodies were applied histologically. To determine oxidative stress, MDA and CAT values were determined. Statistically, one-way ANOVA with post hoc Tukey HSD test for multiple comparisons was performed via IBM SPSS Version 25.0.

Results: Cyclophosphamide caused an increase in testicular MDA levels due to elevated oxidant stress. Testicular MDA levels significantly decreased in zinc and Resveratrol groups which revealed protective effects related to Cyclophosphamide treatment, while no significant improvement was observed for control and saline groups. However, the most significant decrease was observed in MDA for Cyclophosphamide+zinc+Resveratrol group in comparison to Cyclophosphamide. Telocytes, which are lately defined novel cells, were detected in the interstitium encircling seminiferous tubules as a sheath immunohistochemically.

Conclusion: Not only Resveratrol and zinc, but also their optimum administration separately protects testes in Cyclophosphamide treatment groups. Clinical adaptations of this *in vivo* model may lead to novel futuristic ideas in preventing infertility due to cancer chemotherapy.

Keywords: Cyclophosphamide, Resveratrol, zinc, testis.

ÖZ

Amaç: *Siklofosfamid'in neden olduğu deneysel testis hasarında Resveratrol'ün olası koruyucu etkilerini çinko ile kombine ya da kıyaslamalı olarak araştırılması amaçlanmıştır.*

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Gereç ve Yöntem: *Sıçanlar (n=63) rastgele 9 gruba ayrıldı. 21 günlük ilaç uygulaması sonunda; tüm gruplar biyokimyasal ve histolojik olarak incelendi. Günlük su tüketimleri ile deneklerin vücut ağırlıkları ve testis ağırlıkları ölçüldü. Johnsen'in testis skorlaması ve sperm morfolojileri değerlendirildi. Histolojik olarak Hematoksilen&Eozin, Periyodik Asit-Schiff ve Masson'un Trikrom boyaları ve iNOS, eNOS ve CD34 antikorları uygulandı. Doku oksidatif stres düzeyleri için MDA ve CAT değerleri biyokimyasal olarak saptandı. İstatistiksel değerlendirmelerde çoklu karşılaştırmalar için post hoc Tukey HSD testi ile tek yönlü ANOVA, IBM SPSS Sürüm 25.0 kullanıldı.*

Bulgular: *Siklofosfamid, yüksek oksidan stres nedeniyle testis MDA düzeylerini arttırdı. Siklofosfamid tedavisine bağlı koruyucu etkiler ortaya çıkaran çinko ve Resveratrol gruplarında testis MDA seviyelerinde anlamlı düşüşe karşılık, kontrol ve salin gruplarında anlamlı bir iyileşme gözlenmedi. Ancak MDA'da en belirgin azalma Siklofosfamid'e göre Siklofosfamid+çinko+Resveratrol grubunda saptandı. Son zamanlarda yeni tanımlanan hücreler olan telositlerin interstisyumda kılıf şeklinde seminifer tübülleri çevreledikleri immünohistokimyasal olarak gösterildi.*

Sonuç: *Siklofosfamid tedavisi altında testis dokularını korumada sadece Resveratrol ve çinkonun kombine kullanımı değil, optimum dozlarda ayrı ayrı uygulamaları da değerlidir. Bu in vivo modelin klinik uyarlamaları, kanser tedavisine bağlı potansiyel infertilitenin önlenmesinde yeni fikirlerin gelişmesine gelecekte öncülük edebilir.*

Anahtar Kelimeler: *Siklofosfamid, Resveratrol, çinko, testis.*

INTRODUCTION

Cancer has been a frequent cause of death for ages. Novel medications based on molecular mechanisms recently evolved in cancer treatment (1). Cyclophosphamide (CP) is an alkylating antineoplastic, commonly used in chemotherapy (2–4). Inhibition of mitotic activity reveals the cytotoxic and immunosuppressive properties of CP. It reduces adhesion molecules and cytokine production while inducing apoptosis. Lymphocyte number and function abnormalities are observed after CP administration. In addition to acute side effects, gonadal dysfunction may occur in young males where its incidence depends on patient's age, sex, and cumulative exposure (5,6). The severity of the disease, duration of treatment and route of administration may each cause toxicity. Effective and reliable cytoprotective antioxidants are required to prevent chemotoxicity (7–10).

Resveratrol (RES) exhibits its antioxidant role by regulating anti-inflammatory cytokines and is therefore potentially approved as an antioxidant adjuvant in the remedy of many diseases including inflammation, cardiovascular diseases, cancer, etc (11–16). It is difficult to determine in which mechanism oxidative stress is reduced by RES (17). RES has been lately documented to reveal superiority on semen parameters, sperm production and testosterone levels (18–20). Other studies also presented that RES was profitable against testicular damage and sperm

development disorders due to antineoplastics, ischemia-reperfusion injury, etc (21,22). RES increases the production of SOD and CAT through many signaling pathways (23).

Zinc (Zn) is one of the trace elements necessary for stabilization of proteins whose anti-inflammatory, anti-apoptotic and antioxidant effects are already appointed (24, 25) among various biological functions. Thus, Zn ensures the stability of cell membrane against oxidative stress induced by free radicals (26). Also, Zn has roles in sperm maturation by affecting testis and epididymis functions, and regulating testosterone levels (27, 28).

Telocytes (TC) are defined as a novel type of interstitial cells, characterized by a small cell body with very long and thin telopode (Tps) like structures (29). TCs can be easily confused with fibroblasts and muscle cells. TCs create 3D structures by connecting with other cells and provide mechanical support to tissues. In the rat mesentery, TCs were detected to join neighboring cells via desmosomes in order to create a 3D network (30). TCs emit different vesicles and exosomes from Tps, transmit signals and regulate physiological and pathological procedures in various organs. TCs are reported to reveal a regenerative potential and are involved in cellular communication via Tps. The 3D interstitial network created by TC not only provides mechanical support for progenitor cells, but also supports the proliferation, differentiation, maturation and migration of stem cell pool with juxtacrine or paracrine transitions with its atypical connections (31).

To overcome CP, cellular interactions in testis may affect telocyte functions and morphology.

Therefore, the aim of this study is to reveal histologically whether the testicular damage by CP can be reduced via application of various combinations of RES and Zn.

MATERIALS and METHODS

Ethical approval was obtained from the Local Ethics Committee for Animal Experiments (2018-013/28.02.2018). 8-10 weeks old male Sprague Dawley rats (n:63; 200-250 gr) were fed *ad libitum* in a 12 hour light/dark cycle. Experimental groups and applications are shown in Table-1. During the experiment, daily water consumption, body weights and weight of testes were determined. After 21 days, right testes were dissected and fixed for histology while left testes were allocated for biochemistry.

Table-1. Experimental groups and applications.

Groups	Applications
Control	No treatment was applied.
Saline	1 ml/kg/day saline was applied for 21 days.
CP	Cyclophosphamide (Endoxan, Eczacıbaşı-Baxter) was applied intraperitoneally 150 mg/kg on the 15th and 21st days.
CP + RES	150 mg/kg Cyclophosphamide was applied on 15th and 21st days and 20 mg/kg/day Resveratrol was administered by gavage for 21 days.
RES	20 mg/kg/day Resveratrol applied by gavage for 21 days.
Zn	10 mg/kg/day zinc (Santa Cruz Biotechnology) was applied by mixing to drinking water for 21 days.
CP + Zn	150 mg/kg Cyclophosphamide was applied on 15th and 21st days and 10 mg/kg/day zinc was administered by mixing to drinking water for 21 days.
CP + RES+ Zinc	150 mg/kg cyclophosphamide was applied on 15th and 21st days and 10 mg/kg/day zinc was administered in drinking water and 20 mg/kg/day Resveratrol was administered by gavage for 21 days.
RES + Zn	Zinc (10mg/kg/day) was applied in drinking water and 20 mg/kg/day Resveratrol was administered by gavage for 21 days.

- **Biochemical Analyses**

Samples were homogenized (Braun) at 1000 rpm in an iced beaker by diluting 1:10 (w/v) in phosphate buffer pH 7.0 (0.154 M). Then samples were centrifuged for 15 minutes at 4°C at 3500 rpm and supernatants were used to determine testis tissue Malondialdehyde (MDA) levels and Catalase (CAT) activities. The amount of tissue protein was determined by the Lowry method (32). MDA levels were studied with a modified Yagi method (33,34). 750 µl of tissue homogenates and 750 µl of 10% Trichloroacetic acid were centrifuged at 3000 rpm for 10 minutes. 750 µl of the supernatant was mixed with 750 µl of Thiobarbituric acid and absorbance was read at 532 nm. Absorbance values were used to determine concentration using the standard graph measured by the same method. MDA levels were expressed as the amount of MDA per gram tissue (uM/g tissue).

CAT activities were measured using a spectrophotometric-kinetic method based on the principle of degradation of hydrogen peroxide by tissue catalase enzyme activity (35). 100 µl of tissue homogenate was supplemented to a phosphate buffer containing freshly prepared 30 mM H_2O_2 and the reduction in absorbance at 240 nm was recorded at 15-second intervals for 5 minutes where results were given as U/mg protein enzyme activity.

- **Histochemistry**

After carification, testes were rapidly fixed by modified Davidson's fixative for 48 hours (36), and then taken into neutral buffered formalin for another hour and then left in PBS for the next day. Then routine histological tissue processing was performed. 5 mm thick sections (Leica RM 2145) were taken from paraffin-embedded tissues. Deparaffinized sections stained with Hematoxylin&Eosin (H&E), Masson's Trichrome and Periodic Acid–Schiff (PAS) were photographed with a digital camera (DP72; Olympus, Japan) integrated on a microscope (BX51; Olympus).

- **Immunohistochemical expressions of eNOS, iNOS, and CD34**

Deparaffinized sections were incubated with 10% H_2O_2 (Sigma-Aldrich, USA) for 30 min for endogenous peroxidase blockade. To prevent nonspecific binding, sections were incubated with

Serum Blocking Solution (Histostain®-Plus Bulk Kit, Invitrogen Laboratories) for 1 h at room temperature and washed with PBS. Then, sections were incubated with iNOS (sc-7271), eNOS (sc-376751), and CD34 (sc-7324) primary antibodies (1/200; Santa Cruz, CA, USA) for 24h at +40C. Then sections incubated with Biotinylated Secondary Antibody and Enzyme Conjugate (Histostain®-Plus Bulk Kit, Invitrogen Laboratories CA USA, 1/200). Finally, diaminobenzidine was applied until brown color occurred and then counterstained with Mayer's Hematoxylin (Merck, Darmstadt Germany), and cleared with Xylene and mounted.

- **Sperm Parameters**

Caudal epididymis removed from testis was macerated with fine needles until all sperms were extracted in a petri dish containing 10 ml of PBS on a heating plate at 37°C. The samples were incubated for only 15 minutes. Then carefully shaked and 0.5 ml of the homogeneous suspension were transferred to Falcon tubes and mixed with 2 ml PBS. After centrifugation at 1000 g for 5 minutes, the supernatant was discarded and the pellet was dissolved in 1 ml PBS. Finally 10 µl samples were used for counting sperm on Neubauer chambers on each side as in Wang's calculation system (37).

To evaluate sperm morphology, semen was spread onto the slides prepared for sperm analysis and fixed with methanol for 10 minutes and stained with 1% Eosin-Y (Dako, CA, USA). 200 sperms were counted at 100x magnification under immersion oil and the percentage of abnormal head, neck or tails for sperm were detected (38).

Johnsen's testicular scoring was determined by evaluating 100 tubules from randomly selected H&E stained slides of the same magnification for each subject in all groups (39).

- **Statistical Analysis**

Frequency tables of categorical variables and descriptive statistics of continuous variables were calculated. If the numerical data were distributed normally, one way ANOVA was used to compare means of more than two independent groups, and if there was a difference between groups, Tukey HSD test was used as a post hoc comparison method and paired group comparisons were examined. IBM SPSS Version 25.0 was used for statistical analysis where significance was 0.05 for all hypothesis tests.

RESULTS

Histochemistry

Seminiferous tubules and spermatogenic cells in control and saline groups showed normal morphology. Peritubular cells were separated from the basement and there was only mild disintegration recorded for spermatogonia adjacent to basal lamina in RES, while there was no significance in the Zn group compared to the controls (Figure-1).

PAS staining of these groups showed regular basement membrane morphology around the seminiferous tubules (Figure-2). Testicular capsules for all groups appeared normal in Masson's Trichrome stainings (Figure-3).

In the CP group, loss of spermatogenic cells in the seminiferous tubules with excessive degeneration and segregation were determined. Atrophy was observed, as well as narrowing in tubules. Edema in stromal areas, repletion and hyperemia in capillaries, dissociation, and loss of Leydig cells were determined. Seminiferous tubule cells were detected to be minimum in diameter for CP groups in comparison to other groups, as tubule diameter and thicknesses decreased mostly in CP while T. albuginea revealed a looser structure relatively with increased thickness (Figure-1). PAS staining showed detachment of the seminiferous epithelium, possessing a wavy folded appearance and vacuolization, as well as cellular rupture from basal membrane due to tubular distortion, increased peritubular spaces, interstitial edema, and tubular shrinkage (Figure-2). Masson's Trichrome staining showed declined cellular content of interstitium (Figure-3).

Among CP+RES, CP+Zn and CP+RES+Zn groups, CP+Zn showed closeby properties to controls. While there was degeneration and vacuolization in the CP+RES group, moderate atrophy in seminiferous tubules was detected in CP+RES+Zn compared to others (Figure-1). PAS staining showed tubular shrinkage and a decrease in the basement membrane compared to CP (Figure-2). Masson's Trichrome staining of CP+RES and CP+Zn showed cytotoxicity, including stromal edema and interstitial decrement, and a diminishing stroma. CP+RES+Zn also showed severe interstitial edema and injury with diminishing stroma (Figure-3). Localized clusters of lymphocytes were dispersed in all CP groups.

In the RES+Zn group; low-grade atrophy compared to controls was assessed while seminiferous tubule thickness decreased slightly. Cell loss was common in all tissues except for spermatogonia. Moderate edema and dilatation were observed in stroma, and consequences of these findings need to be further evaluated

(Figure-1). Interestingly, mild degeneration in the seminiferous tubules, rupture and disintegration of the basement membranes were recorded via PAS stainings (Figure-2). Unexpectedly, in Masson's Trichrome staining, stromal edema accompanied interstitial alterations (Figure-3).

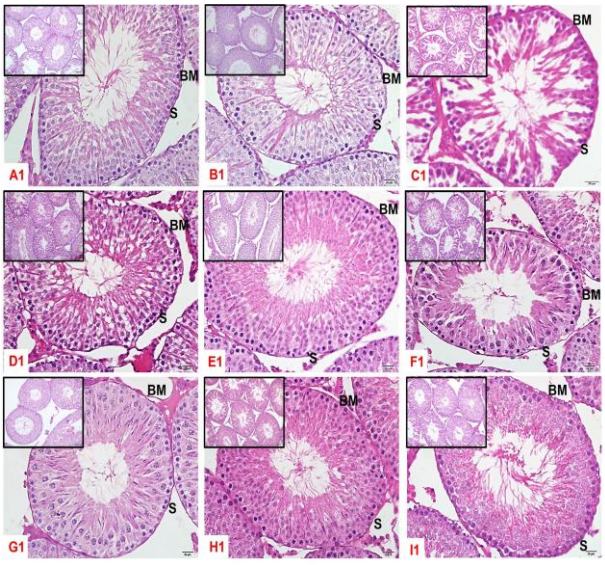

Figure-1. A1-I1 shows H&E stained histological images of the experimental groups. **A1.** Control group. **B1.** Saline group. **C1.** CP group. **D1.** CP+RES group. **E1.** RES group. **F1.** Zn group. **G1.** CP+Zn group. **H1.** CP+RES+Zn group. **I1.** RES+Zn group. BM: Basal membrane structures, S: Spermatogonia. 400x magnification; scale bar: 20 µm.

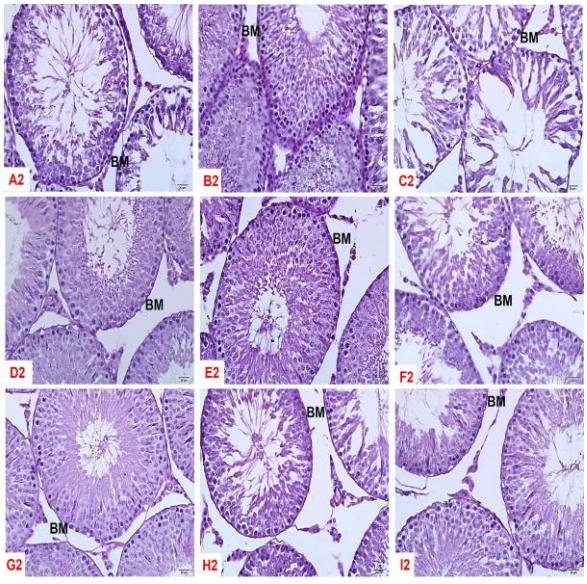

Figure-2. A2-I2 Histological images of the experimental groups with Periodic Acid Schiff (PAS) staining. **A2.** Control group. **B2.** Saline group. **C3.** CP group. **D3.** CP+RES group. **E3.** RES group. **F3.** Zn group. **G3.** CP+Zn group. **H3.** CP+RES+Zn group. **I3.** RES+Zn group. BM: Basal membrane. 400x magnification; scale bar: 20 µm.

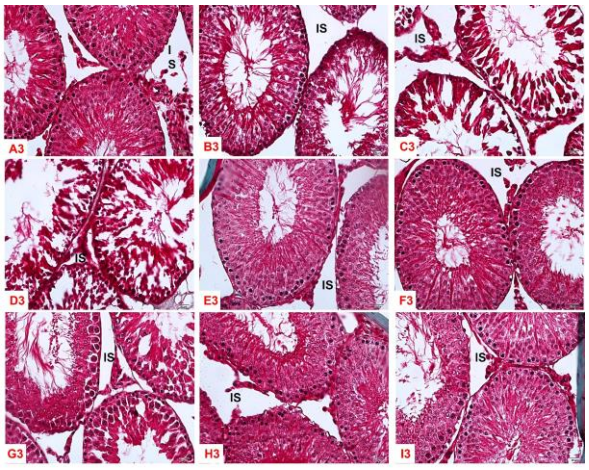

Figure-3. A3-I3 Histological images of the experimental groups with Masson Trichrome staining. **A3.** Control group. **B3.** Saline group. **C3.** CP group. **D3.** CP+RES group. **E3.** RES group. **F3.** Zn group. **G3.** CP+Zn group. **H3.** CP+RES+Zn group. **I3** RES+Zn group. IS: Intesrtitial stromal alterations. 400x magnification; scale bar: 20 µm.

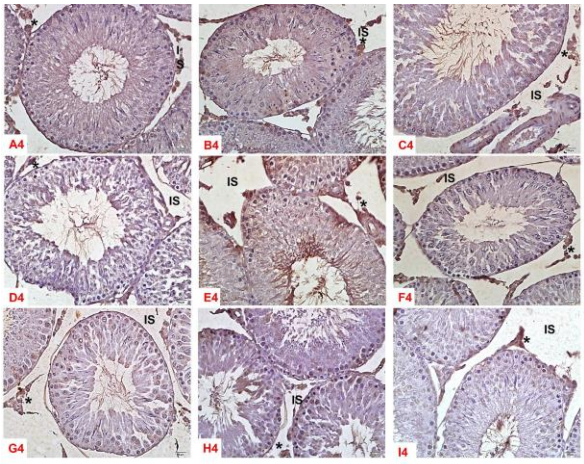

Figure-4. A4-I4 Histological images of the experimental groups with iNOS immunohistochemical staining. **A4.** Control group. **B4.** Saline group. **C4.** CP group. **D4.** CP+RES group. **E4.** RES group. **F4.** Zn group. **G4.** CP+Zn group. **H4.** CP+RES+Zn group. **I4.** RES+Zn group. IS: Interstitial stroma, Leydig cells (*). 400x magnification; scale bar: 20 µm.

Immunohistochemistry

Spermatogenic cells were cytoplasmically moderate to high positive for iNOS staining, although Leydig cells were cytoplasmically high-positive stained in CP. Seminiferous tubules showed various expression profiles, while no difference was recorded for interstitium among all groups (Figure-4). eNOS expression was found in

the cytoplasm of Leydig, Sertoli cells and all spermatogenic cells. Mild stainings were recorded for Sertoli cells, while Leydig cells were medium-high, and endothelial cells were highly cytoplasmic (Figure-5). Telocytes, which are known to be CD34+ cells in interstitium, were cytoplasmically stained and less positive in CP+RES+Zn and RES+Zn (Figure-6). Detailed immunohistological expressions are given in Table-2.

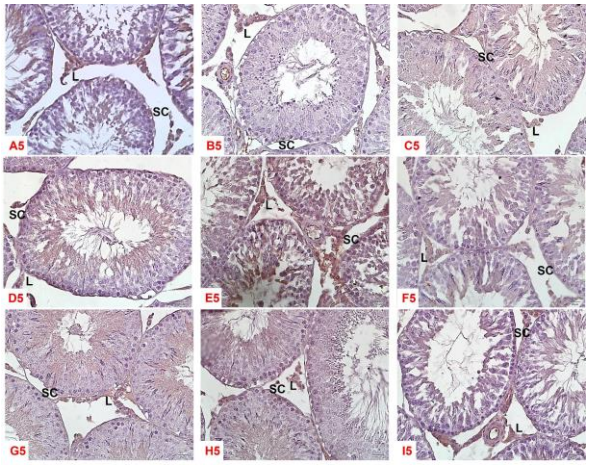

Figure-5. A5-I5 Histological images of the experimental groups with eNOS immunohistochemical staining. **A5.** Control group. **B5.** Saline group. **C5.** CP group. **D5.** CP+RES group. **E5.** RES group. **F5.** Zn group. **G5.** CP+Zn group. **H5.** CP+RES+Zn group. **I5.** RES+Zn group. Leydig (L) and Sertoli cells (SC). 400x magnification; scale bar: 20 µm.

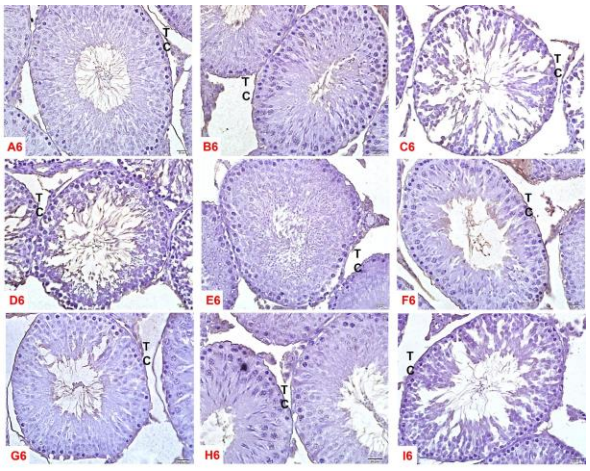

Figure-6. A6-I6 Histological images of the experimental groups with CD34 immunohistochemical staining. **A6.** Control group. **B6.** Saline group. **C6.** CP group. **D6.** CP+RES group. **E6.** RES group. **F6.** Zn group. **G6.** CP+Zn group. **H6.** CP+RES+Zn group. **I6.** RES+Zn group. Telocyte (TC). 400x magnification; scale bar: 20 µm.

Table-2. Histological score and immunoexpression levels of experimental groups.

Immunohistochemical Marker-Region	Control	Saline	CP	CP + RES	RES	Zn	CP + Zn	CP + RES + Zn	RES + Zn
iNOS-Seminiferous tubule	++	++	+++	++	++	++	+++	+++	++
iNOS-Interstitium	++	++	+++	+++	+++	++	+++	+++	+++
eNOS-Seminiferous tubule	+	+	+	++	++	+	+	++	+
eNOS-Interstitium	+++	+++	+++	+++	+++	+++	++	++	+++
CD34-Interstitium	++	++	+++	++	++	++	++	++	+

iNOS: Inducible nitric oxide synthase, eNOS: Endothelial Nitric Oxide Synthase.

Sperm Parameters

In Johnsen's testicular scoring made from H&E-stained sections, a significant difference was observed between CP and Control, Saline, RES, CP+Zn groups. ($p<0.05$). Compared to control and saline, the CP group showed the worst average sperm count scores. When sperm abnormalities were analyzed, CP+RES revealed either shorter tail or no flagella. Other sperm count averages of Zn, RES and CP+Zn groups were likewise. Johnsen's testicular score averages are given in Figure-7.

Biochemical Evaluation

In paired group comparisons of testicular tissue MDA levels (uM/gr wet tissue), CP group MDA levels (24,48±6,97) was higher than CP+RES group (7,18±7,43), Zn group (8,1±5,35) ($p<0,05$) and CP+RES+Zn group (3,82±2,26) ($p<0,01$) while RES+Zn group showed MDA levels (9,14±7,5) not significant than other groups ($p=0,05$, N.S.). Analysis of CAT levels also showed no statistically significant difference ($p>0.05$=N.S.) (Figure-8).

Statistical Evaluation

No statistically significant difference was observed between groups in daily body weight measurements, daily water consumption analyzes and comparisons of testicular weights.

When groups were examined in pairwise comparison using the Tukey HSD method in Johnsen's testicular scoring; statistically significant difference was observed between the CP (7,25±0,19) and Control (8,74±1,03), Saline (8,63±0,95), RES (8,64±0,91) CP+Zn (8,72±0,6) groups ($p<0.05$; Figure-7).

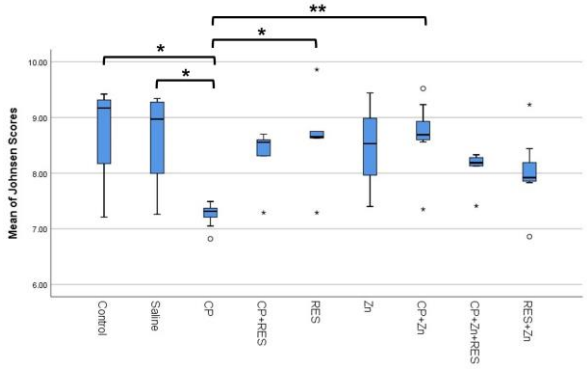

Figure-7. Johnsen's testicular score averages with p values. * $p<0.05$, ** $p<0.005$.

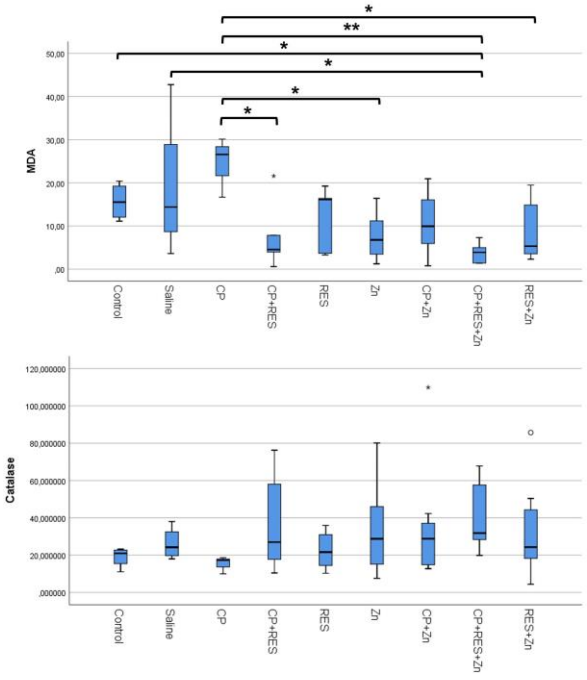

Figure-8. Biochemical analyses between groups.

* $p<0.05$, ** $p<0.005$.

DISCUSSION

Chemotherapy protocols, radiotherapy and surgical interventions constitute current treatments in cancer. Many side effects occur due to chemotherapy, including infertility (40). The use of herbal extracts for pharmacotherapy is one of the successive experimental protocols after chemotherapy. Cyclophosphamide has been used for the treatment of lymphoma, sarcoma and solid tumors, etc. CP has cytotoxic effects in liver, kidney, testis and brain, apart from the target organ which has a tumor (41–45). In accordance with similar studies (46), signs of degeneration such as atrophy in seminiferous tubules and decrease in tubule diameter, loss of spermatogenic cells were observed in our study. In Johnsen testicular scoring, a significant difference was observed between the CP group and others (Figure-7)**.** Similar to the study by Ramos et al., CP had the lowest Johnsen score mean, consistent with its gonadotoxic effects (47, 48). Also, decrease in tubule thickness and loss at spermatogenic level were reflected in the sperm count and morphology of our CP group findings.

RES is documented as an antioxidant drug to reduce DNA damage by decreasing levels for reactive oxygen derivatives (49). El-Sheikh et al. reported effectiveness of RES in multiorgan toxicity resulting from CP administration and RES application luckily reversed the inflammation due to CP (43). Here, in this study, testicular damage due to CP was dispelled due to RES administration for the first time in literature; results of which revealed RES protecting telocyte cells in testis, which appeared to be less disrupted in related groups.

Chronic deficiency of Zn causes hypogonadism (50, 51). In a study by Maremanda et al., effects of Zn administration on testicular damage induced by CP, a decrease in body weight was observed in CP group, which is in line with the results of our study. Accordingly, food consumption and body weights resembled controls after Zn supplementation, while testicular weights were decreased non-significantly in CP (44).

In literature review, administration of both RES and Zn had not been investigated before with CP induced testicular damage. Yet, our RES and Zn

groups lacked significant histopathological findings in comparison to control and saline groups. Zn is a prominent nutritional supplement while RES demonstrated likewise positive effects in the treatment of CP toxicity. Either due to the route or the duration of administration, or even the drug preparation, itself, might have affected the final results. In the review written by Kuršvietienė et al., effects of RES were reported to be dose-dependent while antioxidant properties at low doses became pro-oxidant at higher doses and triggered oxidative stress (52).

Deterioration of the balance between antioxidant and oxidant systems causes tissue damage (53, 54). In the literature, an ischemia-reperfusion model applied in testicular injury models, has been studied (55). It has been reported that RES has positive effects on oxidant/antioxidant balance, improving histopathology while preventing apoptosis in an experimental testicular torsion model (56).

Spermatogenesis is an active meiotic division cycle, with high mitochondrial oxygen consumption in the germinal epithelium. However, poor testicular vascularization causes low oxygen tension. Due to the sensitivity of Leydig cells to oxidative stress for both spermatogenesis and the steroidogenesis, low oxygen possibly protects tissues from free radical damage (57–60).

The antioxidant effects of Zn and RES were evaluated by iNOS and eNOS immunohistochemistry and biochemically MDA and CAT measurements. The fact that the expression of eNOS, which increased in the CP similar to that in the literature, did not regress in the other treatment groups as expected, has resulted in other novel inquiries about either the efficacy of the drugs or optimization of active ingredients of the drugs as antioxidants. The intense expression of eNOS in degenerated early spermatocytes and spermatids indicated its role in spermatogenesis and germ cell degeneration (61).

CP therapy, which decreases intratesticular testosterone concentration, inhibits expression of antioxidant enzymes such as Glutathione peroxidase, Superoxide dismutase and CAT (62–64). Results of MDA analysis in this study revealed that antioxidative therapeutic effects of both RES and Zn applied are significant but not biochemically sufficient.

Localization and morphology of telocytes has been demonstrated in the rat male reproductive system in this study. TCs, first described as a novel interstitial connective tissue cell by Popescu et al. in 2010, have been focused due to their ability to form 3D networks through their morphology. It is valuable to define its structural and functional relationship with spermatogenic cells as well as its interactions with adult stem cell populations in other tissues. TCs were first described in turtles in testicular tissue (65). In addition, recent studies have shown their presence in human, rabbit, rat and mouse testis (66-69). TCs in the testis envelop the seminiferous tubules like a sheath (70). TCs were stained positively with CD34 in rats with testicular injury, for the first time in literature in this study. CD34 is a marker for progenitor cells (71). It was observed that TCs were less expressed in the CP+RES+Zn and RES+Zn groups. This may be due to a competition of drugs. On the other hand, CD34+ expression in all TCs of these experimental groups represent tissue overall regenerative capacity.

CONCLUSION

CP+RES and CP+RES+Zn were applied as drug combinations in rats for the first time in literature. The effects of these drug combinations on telocyte cells in testis were evaluated in the first place. When the blood barrier is disrupted, or in likewise testicular pathologies, this study can architect for the behavior of TCs.

RES was not as successful as Zn in dispelling the toxic effect of CP by crossing the blood testis barrier. Drugs should be redesigned, when their molecular weight and hydrophilic nature are evaluated, such that they easily pass the blood testicular barrier before it is clinically recommended as an adjuvant preparation. RES, which has protective effects in many tissues, may achieve its actual effects in testicular injury as well. Clinical adaptations of this *in vivo* model may reveal other novel clinical ideas for management of potential infertility due to chemotherapy in future.

Conflict of interest: The authors declare that they have no conflict of interest.

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