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RESEARCH

Beneficial role of crocin against doxorubicin-induced testicular damage in rats: insights into vimentin modulation

Sıçanlarda doksorubisin kaynaklı testis hasarına karşı crocinin faydalı rolü: vimentin modülasyonuna ilişkin görüşler

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Abstract

Purpose: Doxorubicin (DOX) is a wide-spectrum antibiotic used for chemotherapy. Its side effects limit treatment. Crocin is one of the carotenoids that has both anti-inflammatory and antioxidant activities. We aimed to evaluate the effects of crocin against doxorubicin-induced testicular damage in rats.

Materials and Methods: Forty Wistar rats were divided into four groups. Group 1: Control, Group 2: Crocin, Group 3: DOX, Group 4: DOX+Crocin (n=10, for all). Testis tissues were stained with Hematoxylin-Eosin. The diameters of seminiferous tubules were measured and the testicular mean histopathologic damage score (MHDS) was calculated. Vimentin expression in Sertoli cells was calculated as H-Score. Levels of Malondialdehyde (MDA), Glutathione (GSH), Catalase (CAT), and Superoxide dismutase (SOD) activities were determined in testis tissues. Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) were also calculated.

Results: Atrophic seminiferous tubules were seen in the DOX group. Edema, vacuolization, and disorganization were present in the injured tubules. The MHDSs for the DOX group and control groups were 4.60±0.45 and 0.20±0.13, respectively. Both of these groups showed a significant difference. The histopathologic score was reduced after using crocin. Tubule damage considerably decreased while immunoexpression levels of vimentin and seminiferous tubule width significantly increased in the DOX+Crocin group compared to the DOX group. MDA and TOS levels were significantly increased after DOX treatment, and GSH, SOD, CAT, and TAS levels were

Öz

Amaç: Doksorubisin (DOX) kemoterapide kullanılan geniş spektrumlu bir antibiyotiktir. Yan etkileri tedaviyi kısıtlamaktadır. Crocin, hem antiinflamatuar hem de antioksidan aktiviteye sahip karotenoidlerden biridir. Sıçanlarda doksorubisin kaynaklı testis hasarına karşı Crosinin etkilerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Kırk adet Wistar sıçan dört gruba ayrıldı. Grup 1: Kontrol, Grup 2: Crosin, Grup 3: DOX, Grup 4: DOX+Crosin (tümü için, n=10). Testis dokuları Hematoksilen-Eozin ile boyandı. Seminifer tübüllerin çapları ölçüldü ve testisin ortalama histopatolojik hasar skoru (MHDS) hesaplandı. Sertoli hücrelerinde Vimentin ekspresyonu H-Score olarak hesaplandı. Testis dokularında Malondialdehit (MDA) ve Glutatyon (GSH), Katalaz (CAT) ve Süperoksit dismutaz (SOD) Kapasitesi düzeyleri belirlendi. Toplam Antioksidan Kapasitesi (TAS) ve Toplam Oksidan Kapasitesi (TOS) da hesaplandı.

Bulgular: DOX grubunda atrofik seminifer tübüller görüldü. Hasarlanan tübüllerde ödem, vakuolizasyon ve düzensizliği mevcuttu. DOX grubu ve kontrol grupları için MHDS'ler sırasıyla 4,60±0,45 ve 0,20±0,13 idi. Bu grupların her ikisi de anlamlı bir fark gösterdi. Crocin kullanımı sonrası histopatolojik hasar skoru azaldı. DOX grubuyla karşılaştırıldığında DOX+Crosin grubunda Vimentin immün ekspresyon düzeyleri ve seminifer tübül genişliği anlamlı derecede artarken tübül hasarı önemli ölçüde azaldı. DOX tedavisi sonrasında MDA ve TOS düzeyleri anlamlı düzeyde artarken, GSH, SOD, CAT ve TAS düzeyleri anlamlı düzeyde azaldı. Crocin aldıktan sonra tüm biyokimyasal göstergeler büyük ölçüde iyileşti.

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significantly decreased. All biochemical indicators were greatly improved after receiving crocin.
Conclusion: Crocin supplementation exhibited adequate beneficial effects against the testicular damage of DOX-induced function by balancing the oxidant/antioxidant status.
Keywords:. Doxorubicin, crocin, testis, vimentin, oxidative stress.

Sonuç: Crosin takviyesi, oksidan/antioksidan durumunu dengeleyerek DOX'un neden olduğu fonksiyonun testis hasarına karşı yararlı etkiler gösterdi.

Anahtar kelimeler: Doksorubisin, crosin, testis, vimentin, oksidatif stres.

INTRODUCTION

One of the most commonly used anticancer drugs in the group of anthracycline antibiotics is Doxorubicin (DOX)[1]. DOX has been shown to exert multiple effects in target cells, including DNA intercalation, induction of DNA breaks, inhibition of topoisomerase II, and formation of toxic free radicals. This cellular toxicity caused by DOX is effective in cancer treatment as a chemotherapeutic agent. However, long-term use is also a concern because of its known side effects on vital organs such as the heart, liver, kidneys, and testis[2-5]. Studies have shown that the most likely causes of DOX-related testicular toxicity are oxidative stress, increased lipid peroxidation products (malondialdehyde), p53-induced increase in apoptosis and decreased DNA synthesis by inhibition of topoisomerase II[2,3,6,7]. It is known that DOX can inhibit spermatogenesis, eventually leading to infertility. Although various agents have studied, no agent has yet been proven to be effective enough to prevent or reverse the testicular damage caused by DOX. Considering the studies showing that different natural product-derived compounds used in studies reverse DOX-induced testicular damage, natural product-derived compounds appear to be the most promising candidates for these adverse effects[5,6,8].

Crocin ($C_{44}H_{64}O_{24}$) is the main pharmacologically active chemical of Crocus sativus L. known as saffron. It is a carotenoid that is rare in nature and easily soluble in water. Carotenoids play an important role in health by acting as natural antioxidants, protecting cells and tissues from the harmful effects of free radicals and reactive oxygen species. Compared to other carotenoids, crocin has a wider application due to its high solubility. It has been used for ages in many countries such as China, Iran, India, and Morocco[9]. In addition, many recent studies have reported that crocin has neuronal and dermal protective, anti-atherosclerotic, anticancer, antihyperlipidemic, antidepressant and antioxidant effects[10-14]. Crocin is one of the carotenoids are thought to be possibly exert chemopreventive effects[15].

DOX is responsible to cause cellular damage especially in Sertoli cells[16]. Previous studies indicated that the chemotherapeutics disrupt the intermediate filaments and seminiferous epithelium[17,18]. Vimentin, as the most common intermediate filament, has functional and structural supportive roles, especially in Sertoli cells, peritubular-myoid cells and Leydig cells[19]. The altered distribution of vimentin in Sertoli cells is associated with several testicular dysfunctions[20].

The protective and therapeutic effects of various antioxidants on DOX-associated testis damage have been evaluated in several studies. However, the potential effects of Crocin on DOX-induced testis damage have not been investigated. Therefore, this study aims to elucidate the protective effects of Crocin against testis damage induced by DOX, a widely used chemotherapeutic agent, using histopathological and biochemical methods.

MATERIALS AND METHODS

Animals

All experimental procedures were performed by the Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee Update of the Guide for the Care and Use of Laboratory Animals Citation 2011). Our animal study protocols and experimental procedures were approved by the ZBEÜN Animal Care and Ethics Committee, as required by law for animal experimentation in Turkey. The authors made the applications with a laboratory animal certificate.

Forty adult male Wistar albino rats (10 weeks age, 225 ±25 g) were purchased from Zonguldak Bulent Ecevit University Faculty of Medicine Experimental Animal Production and Research Center (ZBEÜN-DEHAM). All rats were housed in well-ventilated room with temperature- and humidity-controlled

conditions (an ambient temperature range of 22°C; relative humidity of 55-60%) in rat cages with a 12h-12h light-dark cycle (light from 08:00-20:00). The study was approved by the Experimental Animals Ethics Committee of Zonguldak Bulent Ecevit University, Faculty of Medicine (Protocol No: 2021-09-01/04). All experimental procedures were carried out in accordance with the Animal Ethics Committee Guidelines for the use of experimental animals. The experimental animals were allowed access to drinking water and standard rodent diet *ad libitum*.

Experimental design

DOX (Doxorubicin® 10 mg) was purchased from Kocak Company (Istanbul, TURKEY) and crocin was obtained from Sigma Aldrich Corporation (St. Louis, Missouri, ABD). The number of animals used in the experiment was determined in accordance with the Guide for the Care and Use of Laboratory Animals, local ethics committee recommendations and G-Power analysis. Power analysis was performed using G-Power 3.1.9.7. With an effect size of 0.5, an alpha margin of error of 0.05, and a power of 70%, the total number of experimental animals for the 4 groups was 40. Forty Wistar rats were equally randomly (completely randomized experimental design) divided into 4 groups. Each experimental group consisted of 10 animals. Group 1 (Control): Animals recevied normal saline (1 ml/kg) via intraperitoneal injection (i.p.) for 15 days. Group 2 (Crocin): Animals received crocin (40 mg/kg) via i.p. for 15 consecutive days. Group 3 (DOX): Animals received DOX (2 mg/kg) via i.p. in six injection at 48 hours intervals during the 12-day period (cumulative dose:12 mg/kg). Group 4 (DOX+Crocin): Animals received crocin (40 mg/kg) through 15 consecutive days (starting 4 days before first DOX administration) along with DOX treatment (with the same dose as the group mentioned above). Lyophilized DOX powder was prepared for i.p. injection by dissolving with the solvent water. Crocin powder was dissolved by normal saline (0.9%). Throughout the study, all applications were carried out everyday in the same time period (12:00-13:00 PM). The chemicals used in this study were determined according to the literature review[21,22].

Tissue collection

At the 16th day of the experiment, all rats were anesthetized under ketamine/xylazine anesthesia. The rats were then sacrificed by cervical decapitation and the testes were removed immediately. Right testes were stored at -80°C until for measurement of biochemical parameters. Left testes were fixed in 10% neutral buffered formalin for histological examinations.

Histological analysis

Histopathological evaluation

The testis tissues were fixed in 10% neutral buffered formalin for 48h. Tissues were washed in running water, and were dehydrated with increasing concentrations of ethanol (50%, 75%, 96% and 100%). After dehydration, specimens were placed into xylene to obtain transparency and were embedded in paraffin. Paraffin blocks were cut at 5 μm, mounted on slides, stained with hematoxylin and eosin (H-E). The tissue sections were examined under light microscopy. The diameters of seminiferous tubules were measured and the damage was evaluated. The evaluated parameters for severity of testicular damage were atrophy, edema, vacuolization and disorganization of seminiferous tubules in 10 different fields for each section. For this analysis, testicular damage was semiquantitatively graded as absent (0), mild (1), moderate (2), and severe (3), for each criterion. The maximum mean histopathological damage score (MHDS) was 12. The evaluation was done in a double-blind fashion in the study. All sections were examined using a Nikon Eclipse 80i light microscope and Nikon Image Analysis system.

Immunohistochemical (IHC) evaluation

For immunohistochemical analysis, sections were mounted on polylysine coated slides. After deparaffinization, samples were transferred to citrate buffer (pH 7.6) and heated in a microwave oven for 20 min. After cooling for 20 minutes at room temperature, the sections were washed with phosphate buffered saline (PBS). Then sections were kept in 0.3% H_2O_2 for 7 min and afterward washed with PBS. Sections were incubated with an anti-Vimentin antibody (1:100 dilution, rabbit polyclonal, Catalog No: FNab08038, Lot No: 20191025, FineTest, China) for 60 min. They then were rinsed in PBS and incubated with biotinylated goat antipolyvalent for 10 min and streptavidin peroxidase for 10 min at room temperature. Staining was completed with chromogen+substrate for 15 min, and slides were counterstained with Mayer's hematoxylin for 1 min, rinsed in tap water, and

dehydrated. The antibody was used according to the manufacturer's instructions. Staining for anti-Vimentin was identified by a brown color. The relative intensity of vimentin immunostaining was scored as follows: (0), (1), (2) or (3) (no-stained, mild, moderate or strong respectively). Semi-quantitative histo-score (H-SCORE) values were calculated (H-Score = ∑Pi (i+1), i; the relative intensity of staining and Pi; the percentage of cells stained with each intensity, varying between 0-100%)[23]. All sections were examined using a Nikon Eclipse 80i light microscope and Nikon Image Analysis system.

Biochemical analysis

Preparation of samples

Testis tissues were removed from the freezer and rapidly weighed. Then the tissues were homogenized at 10,000 rpm for one minute by an automatic homogenizer (Bioprep-24 Homogenizer, Hangzhou Allsheng Instruments Co., Ltd., China) in a 10-fold volume ice-cold phosphate buffer (0.001 M, pH 7.4). The homogenates were used for determination of Malondialdehyde (MDA) analysis. To obtain supernatant, the homogenates were centrifuged at +4°C and 3000 rpm for 10 min. The supernatants were used for determination of tissue biochemical parameters. The evaluation was done in a double-blind fashion in the study.

Assessment of oxidative stress markers

Testis tissue homogenate was used for assessment of lipid peroxidation by measuring testicular MDA levels according to the method of Ohkawa *et al.* (1979)[24]. Testis tissue supernatants were used for the determination of the antioxidant enzyme activities. Testicular superoxide dismutase (SOD) activity was determined with the method proposed by Sun *et al.* (1988)[25], and testicular catalase (CAT) activity was determined by the method of Aebi (1974)[26]. Reduced glutathione (GSH) content, one of the non-enzymatic antioxidant markers, was determined with the method illustrated by Ellman (1959)[27]. Testicular protein content was assessed according to the Biuret method Gornall (1949) using bovine serum albumin (BSA) as standard and used for calculating the antioxidant enzyme's activities[28]. Total antioxidant status (TAS) of testicular tissue was determined with the method proposed by Erel (2004)[29]. TAS was performed according to the manufacturer's instructions (Rel Assay Diagnostics, Gaziantep, Turkey). Total oxidant status (TOS) of testicular tissue was measured according to the method of Erel (2005)[30]. TOS was performed according to the manufacturer's instructions (Rel Assay Diagnostics, Gaziantep, Turkey). The evaluation was done in a double-blind fashion in the study.

Statistical analysis

Statistical analysis was carried out using the SPSS for Windows version 14.0 (SPSS Inc., Chicago, III., USA) statistical program. All data are expressed as arithmetic mean ± SE. Normality for continued variables in groups were determined by the Shapiro Wilk test. The variables didn't show normal distribution ($p<0.05$). Because it does not comply with the normal distribution, Kruskal-Wallis (Tamhane's test for paired comparisons of groups) and Mann-Whitney U tests were used for the comparison of variables among the studied groups. $p<0.05$ was regarded as significant.

RESULTS

Control group showed normal histological structure (Figure 1A). The crocin group showed a similar pattern to the control group (Figure 1B). In group 3 which is treated with DOX, it was detected atrophied seminiferous tubules in many areas with disorganization, vacuolization, and edema (Figure 1C). The mean histopathological damage scores (MHDSs) of each group were calculated as 0.20±0.13 (Group 1), 0.30±0.15 (Group 2), 4.60±0.45 (Group 3), and 2.60±0.16 (Group 4) (Figure 1). The MHDS of DOX group was significantly different with the control and crocin group ($p<0.001$). MHDSs of DOX+Crocin group were seen significantly decreased compared to DOX group ($p<0.001$).

Crocin administration decreased disorganized testicular damage (Figure 1D) in parallel with the MHDS. In addition, it was determined that the diameters of seminiferous tubules in DOX group (21.48±0.98 μm) was decreased compared to the control (38.57±1.50 μm) and crocin group (36.76±3.56 μm) ($p<0.001$) (Figure 1). In the DOX+Crocin group, the diameters of seminiferous tubules (30.23±1.42 μm) significantly increased to the DOX group ($p<0.001$) (Figure 1).

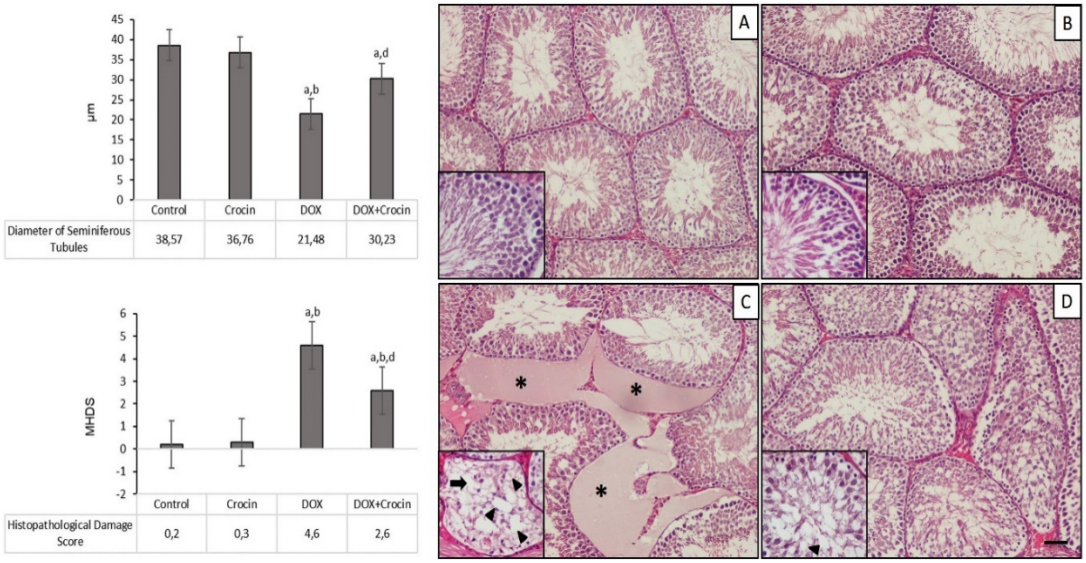


Figure 1. The photomicrographs of histopathological analysis. Control (A) and crocin (B) groups showed normal seminiferous tubules (t) and interstitium (i). DOX (C) group showed tubular atrophy (black arrow), tubular vacuolation (arrow head) and interstitial edema (*). DOX+Crocin (D) group showed decrease in tubular atrophy (black arrow) and tubular vacuolation (arrow head). İnterstitial edema was not observed in this group. A. Group 1: Control, B. Group 2: Crocin, C. Group 3: DOX, D. Group 4: DOX+Crocin.

The magnification for bigger images is H-E; X10 and for bottom left images is H-E; X40. Bar: 20 µm. All data are expressed as arithmetic mean±SE (n=10). [a] p<0.001 Control group, [b] p<0.001 Crocin group, [c] p<0.005 Control group; [d] p<0.001 DOX group.

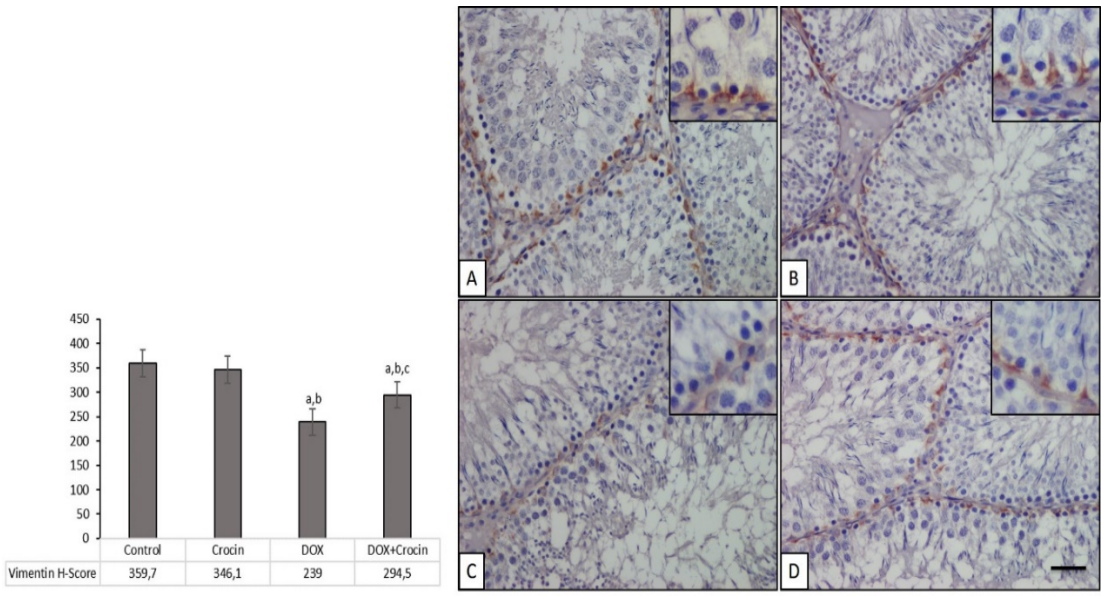


Figure 2. The photomicrographs of Vimentin immunoexpression. A. Group 1: Control, B. Group 2: Crocin, C. Group 3: DOX, D. Group 4: DOX+Crocin.

The magnification for bigger images is; X10 and for bottom left images is; X40. Bar: 20 µm. All data are expressed as arithmetic mean±SE (n=10). [a] p<0.001 Control group, [b]p<0.001 Crocin group, [c] p<0.001 DOX group.

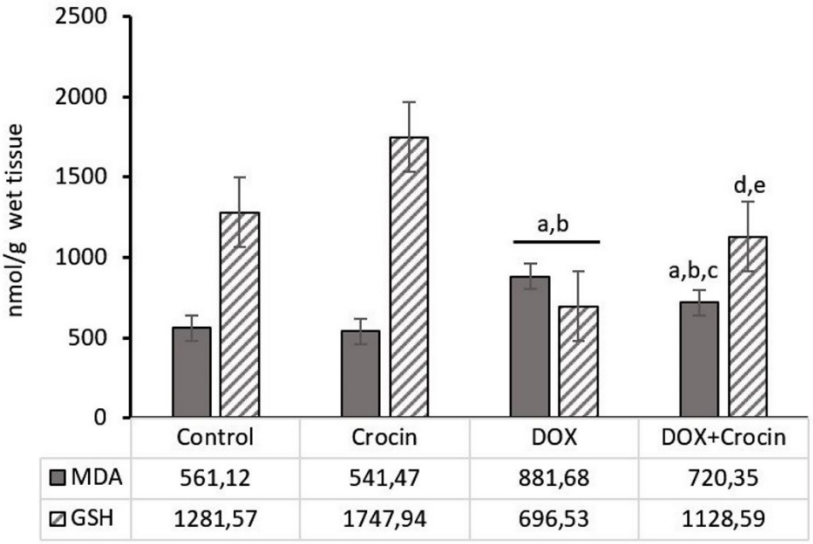
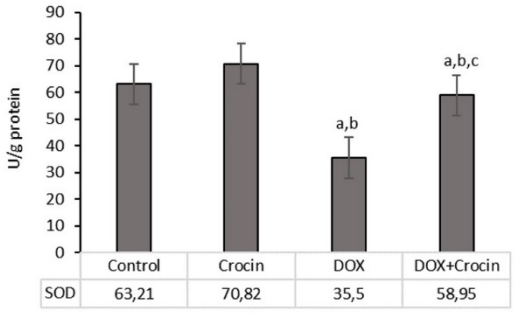
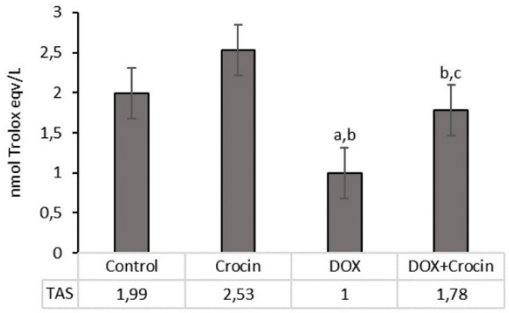
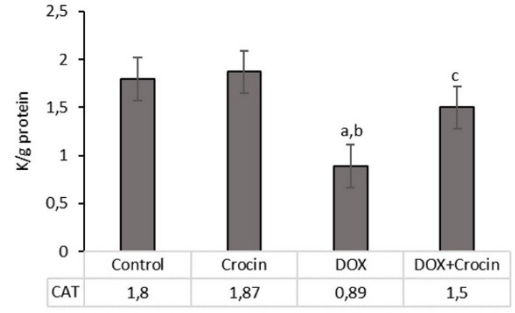


Figure 3. Results of tissue MDA and GSH levels.

All data are expressed as arithmetic mean±SE (n=10). [a] p<0.001 Control group, [b] p<0.001 Crocin group, [c] p<0.005 DOX group, [d] p<0.05 Control group, [e] p<0.001 DOX group.







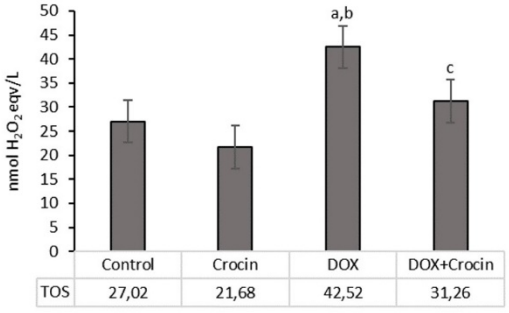


Figure 4. Results of tissue SOD and CAT activities. All data are expressed as arithmetic mean±SE (n=10). a p<0.001 Control group, b p<0.001 Crocin group, c p<0.001 DOX group.

Figure 5. Results of tissue TAS and TOS levels. All data are expressed as arithmetic mean±SE (n=10). a p<0.001 Control group, bp<0.001 Crocin group, cp<0.005 DOX group, dp<0.05 Control group, ep<0.001 DOX group.

The presence of vimentin was observed only in Sertoli cells in testis tissues of rats by immunohistochemical staining (Figure 2). It was determined that vimentin immunoreactivities of Sertoli cells were strongly positive in control group (Figure 2A) and crocin group (Figure 2B). The immunoreactivity of vimentin was observed decreased in DOX group (Figure 2C) and DOX+Crocin group (Figure 2D). It was calculated that the vimentin H-Score of Sertoli cells in DOX group was significantly reduced compared to control and crocin group ($p<0.001$) (Figure 2). In DOX+Crocin group, the vimentin H-score was significantly decreased in rat Sertoli cells compared to DOX group ($p<0.001$) (Figure 2)

The values of MDA, GSH, antioxidant enzymes (SOD, CAT), TAS, and TOS were analyzed biochemically. The levels of each parameters showed no statistically significant changes between control and crocin groups. MDA and TOS levels for DOX group were increased significantly compared to control group ($p<0.001$). In the DOX+Crocin group, MDA and TOS levels were significantly decreased compared to DOX group ($p<0.001$) (Figures 3, 5). The GSH level decreased in DOX group compared to control group ($p<0.001$) and increased in the DOX+Crocin group compared to DOX group ($p<0.001$) (Figure 3). The activities of antioxidant enzymes (SOD, CAT) was seen as decreased in DOX group compared to the control group ($p<0.001$) and increased in the DOX+Crocin group compared to the DOX group significantly ($p<0.001$) (Figure 4). Similarly, in the DOX group, TAS levels also revealed significant reductions compared to control group ($p<0.001$) and significant increase in the DOX+Crocin group compared to DOX group ($p<0.001$) (Figure 5).

DISCUSSION

Testicular dysfunction is one of the main side effects of chemotherapy[31]. Doxorubicin (DOX) as an anticancer drug has been reported to have toxicity on male reproductive system. DOX causes the chromosome abnormalities on cells and changes the testicular lipid metabolism negatively[32]. Sertoli cells are responsible to control spermatogenesis, crosstalk with Leydig and myoid cells and maintain blood-testis barrier[33]. In several in vitro studies showed that the DOX-induced toxicity causes the apoptotic cell death and induces oxidative stress on Sertoli cells[34,16]. It was also showed in rodent models that DOX affects the spermatogenesis through the reducing the testosterone levels, sperm count and sperm motility and causes irreversible testicular toxicity[31,35]. Moreover, it is also known that doxorubicin-containing regimens increase the frequency of aneuploid sperm[36].

Many clinical and experimental studies showed that DOX impairs male reproductive function. Light microscopic evaluation revealed testicular damage comprising various degrees of seminiferous tubule degeneration after DOX treatment. Ozturk et al. (2020) reported that DOX treatment causes the decrease of body weight and testis weight of rats and the diameters of seminiferous tubules were significantly decreased[37]. They also observed the disorganization of seminiferous epithelium, vacuolization and decrease of germinal cells. In another study, the testicular diameters are reported significantly lower after the DOX treatment to the control rats. It was also observed disrupted spermatogenesis and separated germ cells from the basal membrane in testis tissues of DOX groups[17]. The high vacuolization and irregular basement membrane structure is commonly observed after DOX applications[18]. Lee et al. (2012) found that testicular tissue showed extensive injuries characterized by decrease of spermatogonia, degeneration and/or decrease of early spermatocytes, and vacuolated seminiferous epithelium[38]. Our results show that DOX causes vacuolization, edema and disorganization in seminiferous tubules and there is germ cell loss in seminiferous tubules. These histopathological findings in the seminiferous tubule epithelium may decrease sperm concentration and motility. Sperm motility is one of the important parameters for fertilization and any positive effect on motility can alter fertilization capacity.

Vimentin plays a role in anchoring germ cells to the seminiferous epithelium and controls spermatid movement. Vimentin filament damage is associated with the seminiferous epithelium disintegration and disruption of spermatogenesis[20]. The previous studies showed that under the effects of anti-cancer drugs the vimentin distributions is disrupted and the vimentin filaments re-organized in perinuclear or basal areas of Sertoli cells[20,39]. In our results it is seen that the DOX administration was also decreased the intensity of vimentin in Sertoli cells. The alteration of

vimentin in Sertoli cells under the influence of DOX may cause weakening of the blood-testis barrier and may affect the spermatogenesis process, which may lead to infertility.

MDA is a polyunsaturated fatty acid peroxidation product and an accepted indicator of oxidative stress[40]. The MDA concentration increases under oxidative stress[41]. Potential oxidative stress sources lead to a decrease in tissue GSH levels, decrease in antioxidant enzyme activities and increase ROS production[42]. El-Maddawy *et al.* (2019) reported that in DOX-treated group the GSH and CAT levels were significantly decreased, and MDA level was significantly increased[43]. Moreover, Huyut *et al.* (2020) showed the serum MDA levels were significantly higher in DOX groups[17]. In our study, it was found that DOX treatment causes the decreasing of GSH and CAT and increasing of MDA similarly in testis. Decreased GSH and CAT activities and a significant increase in the testicular MDA concentration are associated with increased oxidative stress. The induced oxidative stress by DOX can explain the disorganization, vacuolization, and edema of testicular histology and disrupted vimentin distribution on Sertoli cells. As an internal antioxidant enzyme, the SOD activity reported significantly decreased in DOX group compared to control group[44,45]. SOD activity shows the conversion of superoxide radicals and protection of cell from oxidative stress[45]. The lower levels of SOD mean that the DOX causes oxidative toxicity in testis. It was reported in a study of DOX-treated rats, total antioxidant status (TAS) is significantly decreased and total oxidant status (TOS) was significantly increased in testicular tissue compared to control group[37]. Our results showed the similar pattern after the DOX administration. These alterations of TAS and TOS levels are suggested that the DOX play a crucial role to induce testicular oxidative damage. Together these parameters clearly indicate that the testis is highly exposed to DOX-induced toxicity and that oxidative stress occurs.

Crocin is water soluble carotenoid as an active compound of saffron known with its antioxidant, anti-inflammatory, anti-apoptosis and protective effects on DNA damage[46]. Crocin is a carotenoid that is rare in nature. Carotenoids are essential for good health because they act as natural antioxidants, protecting cells and tissues from the harmful effects of free radicals and ROS. In comparison to other carotenoids, crocin has a wider range of applications due to its high solubility.[9] In many experimental toxicology studies, it has been shown that crocin has a healing effects on testis[47,48]. In addition, crocin has been shown to have beneficial effects against DOX-induced myocardial toxicity[21,49,50]. Our histological findings showed that crocin has a recovery effect on seminiferous tubules. The number of vacuolized areas and the MHDS were lower in DOX+Crocin group compared to DOX-treated group. Moreover, the diameter of seminiferous tubules was significantly higher. The crocin has been reported with its protective or healing effects in rodent models against to electromagnetic field induced testicular[51], streptozotocin-induced diabetic damage[52] and paraquat-induced oxidative stress[53]. In all these studies, the histopathologic evaluations of testis tissues showed improved structures after the Crocin treatment. However, in the literature, there is no any histological data or any information about vimentin distribution on DOX-induced testicular damage after the crocin treatment. This is the first study to show that DOX disrupts the distribution of vimentin in Sertoli cells and that crocin treatment has the capacity to ameliorate this deterioration.

Davoodi et al. (2021) reported that the crocin treatment decrease protein carbonyl (PC) levels, increase glutathione peroxidase (GPX) and total antioxidant capacity (TAC) levels after the DOX-induced testicular toxicity in rats[54]. They state that crocin treatment provides an increase in antioxidant levels and this increase appears to be dose-dependent and also reported the MDA, SOD and CAT levels should be performed additionally[54]. Our results showed that the negatively changed MDA, GSH, SOD, CAT, TAS, and TOS levels after DOX applications become improved levels after the crocin treatment. As a result of biochemical analysis, it was clearly seen that crocin has significant capacity to fight against DOX-induced testicular damage.

Although the current study has produced the expected results, it has some limitations. Validation of the data obtained from electron microscopy studies may make the results even more reliable. Since the consumption of bile is oral, it would have been more appropriate to give crocin, the antioxidant used in our study, to rats via oral gavage.

In conclusion, present study demonstrated that crocin has potential beneficial effects against DOX-induced testicular damage by modulating oxidant and antioxidant systems, and reorganization of vimentin in Sertoli cells which is supported by

histopathological evaluations. Crocin is suggested to be used as a protective or therapeutic agent against DOX-induced testicular damage. The use of DOX and Crocin at different doses and for different durations may allow new studies to determine the most effective treatment dose and recovery period.

Abbreviations: Bovine serum albumin (BSA), Deoxyribose nucleic acid (DNA), Doxorubicin (DOX), MDA (Malondialdehyde), GSH (Glutathione), CAT (Catalase), SOD (Superoxide dismutase), TAS (Total Antioxidant Status), TOS (Total Oxidant Status), MHDS (mean histopathologic damage score).

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