Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2025;50(1):137-147
DOI: 10.17826/cumj.1607896

RESEARCH

Evaluation of the protective and therapeutic effect of *Salvia officinalis* against testicular damage caused by docetaxel in rats

Sıçanlarda dosetakselin neden olduğu testis hasarına karşı *Salvia officinalis*'in koruyucu ve terapötik etkisinin değerlendirilmesi

Mustafa Öztürk[1], Demet Bolat[2], Arzu Yay[2,3], Perihan Gürbüz[4], Münevver Baran[5]

[1]Erciyes University, Kayseri, Türkiye

Abstract

Purpose: This study aims to investigate the possible side effects of the chemotherapeutic agent Docetaxel on the male reproductive system and in vivo the preventive or therapeutic role of the hydroalcoholic extract of *S. officinalis* L. against these effects.

Materials and Methods: In the study, 50 Wistar albino male rats were divided into five groups: Control; Docetaxel; *S. officinalis*; *S. officinalis* + Docetaxel; and Docetaxel + *S. officinalis* groups. After the treatments, the testicular tissues of the rats were excised and fixed in 10% formaldehyde. After routine tissue processing, sections were taken. Then, hematoxylin-eosin and immunohistochemical staining (PGP 9.5 and DAZL1) were performed, and immunoreactivity intensity was evaluated.

Results: In the light microscopic examinations of the preparations to which the routine histological tissue tracking method was applied, the testicular architecture was observed to be normal in the control group, while pathological changes such as irregularity, hemorrhage, and spermatogenic cells spilling into the lumen were observed in the seminiferous tubule epithelium in the docetaxel group. In the evaluation made according to the Johnsen scoring system, a significant decrease was detected in the docetaxel group compared to the control group, and the groups applied only to *S. officinalis*. According to the analysis of the immunoreactivity intensities of PGP 9.5 and DAZL1, it was seen that Sal applied before docetaxel application showed protective properties on the testicular tissue and increased the expression levels compared to the Dox + Sal group

Conclusion: This study shows that *S. officinalis* 70% methanol (MeOH) extract may protect against the negative effects of docetaxel on testicular tissue. Our findings are promising because they indicate that when given to

Öz

Amaç: Bu çalışma ile kemoterapötik ajan olan Dosetakselin erkek üreme sistemi üzerindeki olası yan etkileri ve bu etkilere karşı *S. officinalis* L. bitkisinden hazırlanan hidroalkolik ekstresinin koruyucu veya tedavi edici rolününün in vivo olarak araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmada, 50 adet Wistar albino erkek sıçan beş gruba ayrılmıştır: Kontrol, Dosetaksel, *S. officinalis,* *S. officinalis* + Dosetaksel ve Dosetaksel + *S. officinalis* grubu. Uygulamalar sonrasında sıçanların testis dokuları eksize edilmiş ve %10'luk formaldehitte fikse edilmiştir. Rutin doku takip işlemlerinin ardından kesitler alınmıştır. Daha sonra Hematoksilen-Eozin ve immünohistokimyasal boyamalar (PGP 9,5 ve DAZL1) yapılmış ve immunreaktivite yoğunlukları değerlendirilmiştir.

Bulgular: Rutin histolojik doku takip yöntemi uygulanan preparatların ışık mikroskobik incelemelerinde kontrol grubunda testis mimarisinin normal olduğu gözlenirken, docetaxel grubunda seminifer tübül epitelinde düzensizlik, hemoraji ve spermatogenik hücrelerin lümene dökülmesi gibi patolojik değişiklikler izlendi. Johnsen skorlama sistemine göre yapılan değerlendirmede docetaxel grubunda kontrol grubuna ve sadece *S. officinalis* uygulanan gruplara göre anlamlı azalma saptand). PGP 9.5 ve DAZL1'in immunoreaktivite yoğunluklarının analizine göre docetaxel uygulamasından önce uygulanan Sal'ın testis dokusu üzerinde koruyucu özellik gösterdiği ve Dox + Sal grubuna göre ekspresyon düzeylerini artırdığı görüldü .

Sonuç: Bu çalışma, *S. officinalis* %70 metanol (MeOH) ekstresinin docetaxel'in testis dokusu üzerindeki olumsuz etkilerine karşı koruma sağlayabileceğini göstermektedir. Bulgularımız umut vericidir çünkü dosetaxel uygulamasından önce hastalara verildiğinde, *S. officinalis'in* belirgin koruyucu özelliklerinin yeni bir tedavi seçeneği sağlayabileceğini göstermektedir. Ayrıca, *S. officinalis*'in

Address for Correspondence: Munevver Baran, Department of Basic Sciences, Faculty of Pharmacy, Erciyes University, Kayseri, Türkiye E-mail: munevverbaran@erciyes.edu.tr
Received: 31.12.2024 Accepted: 11.03.2025

patients before dosetaxel administration, the distinct protective characteristics of *S. officinalis* may provide a novel therapeutic option. It is also thought that it may contribute to future in vitro studies examining the effects of *S. officinalis* on the reproductive system.
Keywords: Testis, docetaxel, *Salvia officinalis*, DAZL1, PGP 9,5

üreme sistemi üzerindeki etkilerini inceleyen gelecekteki in vitro çalışmalara katkıda bulunabileceği düşünülmektedir.

Anahtar kelimeler: Testis, dosetaksel, *Salvia officinalis*, DAZL1, PGP 9,5

INTRODUCTION

Genital malignancies (such as testicular germ cell tumors and prostate) have a substantial impact on men as they include the most common cancers. Apoptosis suppression, aberrant, unchecked cell proliferation, and the propensity for metastasis are characteristics of cancer. One of the main causes of death for people in the past century, cancer is traditionally treated with surgery, radiation, and chemotherapy. While improvements in these techniques have greatly raised the success rate of cancer treatment, chemotherapy procedures, in particular, that use strong cytotoxic medicines, can have major side effects and damage nontargeted organ tissue. One of the most frequent adverse effects of chemotherapy medications is testicular toxicity. Several studies have suggested that chemotherapeutics may have a major impact on spermatogenesis and male fertility[1,2].

Antimicrotubule chemotherapeutic drugs belonging to the taxane group are frequently used to treat prostate, kidney, and lung cancers[3]. One of the important chemotherapeutic drugs in this group is docetaxel (Dox), which is one of the most effective agents, especially in the treatment of prostate cancer[4,5]. It has been reported that Dox may cause various side effects on healthy rat testicular tissues after application[6,7]. However, there is still insufficient information on the nephrotoxicity and testicular toxicity of Dox. According to certain research, Dox toxicity is linked to an excess of reactive oxygen species[6,7,8].

Therefore, natural resources that can successfully reverse this negative situation are being investigated. Today, medicinal plants are considered a source of effective biologically active compounds[9] and exhibit pleiotropic effects on organisms, with high potential for therapeutic use. *Salvia* is the largest genus in sage family Lamiaceae, and *S. officinalis* (common sage), is a notable medicinal plant widely distributed all over the world[10]. Bioactive compounds of sage are declared to be phenolic compounds, i.e., phenolic acid and its derivatives, flavonoids, and terpenoids comprising di- and triterpenes[11]. According to Alshubaily and Jambi, sage, which is an aromatic medicinal plant, is used to treat several ailments, such as diabetes, cancer, heart disease, reproductive problems, and hypercholesterolemia.

Phytochemicals and their derivatives possess anti-cancer properties, including the potent ability to modulate cell signaling. It is proved that a successful therapeutic strategy to manage cancer is achieved by simultaneously targeting intracellular signaling pathways associated with carcinogenesis using different bioactive compounds found in medicinal plants[12]. Phytochemicals have the ability to limit cancer development and spread by affecting processes such as cell cycle, programmed cell death, new vessel formation, stem cell activity and metastasis, and with these properties, they may exhibit potential oncostatic effects[12]. Numerous chemotherapeutic agents can penetrate the blood-testis barrier and harm germ cells irreversibly. Due to their high mitotic activity, germinal epithelial cells are susceptible to chemotherapeutics. If stem cells are not harmed by chemotherapy, spermatogenesis might resume in a specific amount of time[13]. To boost male fertility, a variety of bioactive phytochemicals have been employed[14]. The large array of potential bioactive phytoconstituents is thought to have an impact on metabolic activity, the generation of reactive oxygen species, steroid hormone production, and the initiation of reproductive health changes[14,15]. The potential effects of *S. officinalis* on testis tissue are mostly unknown. The hypothesis is to compare the therapeutic and protective effects of *S. officinalis* in the treatment of Dox-induced testicular damage. This study demonstrated for the first time that pre-and post-treatment with *S. officinalis* attenuated Dox-induced damage to the rat testis. This study aimed to reveal the possible effects of Dox, which is widely used in cancer treatment, on testicular tissue and cells using histopathological and immunohistochemical methods and to investigate the preventive or therapeutic effects of *S. officinalis* to reduce or prevent these effects. The results of the study will provide a

new perspective to the literature on the prevention of damage to the Dox caused by *S. officinalis*.

MATERIALS AND METHODS

Plant material and sample preparation

Salvia officinalis L. grown with organic farming methods was obtained in May 2021 from Temmuz Organik Çiftliği in Konya, Türkiye (Beyşehir Road, 3 km Akyokuş Mevki; Certificate No. TR-OT-014-Ü-197/01). This species was grown in accordance with the Organic Agriculture Law and Regulation of the Turkish Republic and was certified by Nissert (Ankara, Türkiye) as authorized by the Ministry of Agriculture.

The dried and powdered plant material (100 g) was extracted with 3 x 1 L of 70% MeOH at 37 °C. Following the evaporation of the solvents under vacuum, 25 grams of crude MeOH extract (25% yield) were obtained.

Animals and experimental protocol

The study was approved by the Erciyes University Animal Experiments Local Ethics Committee (Decision No: 21/164), and the experimental procedures involving animals were conducted at the Erciyes University Experimental Research Application and Research Center. The rats were housed in specially designed, automatically air-conditioned chambers with 12-hour light/dark cycles and a consistent temperature of 19–21 ºC throughout the trial. Each cage was placed with 4 rats and fed freely with normal pellet-type food and water. In our study, 50 Wistar albino male rats (8–10 weeks old, 200-250 g) were randomly selected, and 5 experimental groups (n=10) were created.

Control group: Only distilled water (1 mg/kg) was administered orally for 5 days. Dox group on the first day, 30 mg/kg dox was administered as a single dose intraperitoneally (i.p.)[16]. *S. officinalis* group (Sal): The group administered *S. officinalis* (100 mg/kg) orally for 5 days[17]. Dox + *S. officinalis* group (Dox + Sal): After the single dose i.p. of dox at a dose of 30 mg/kg on the first day, 30 minutes later, *S. officinalis* was administered orally at a dose of 100 mg/kg/day for 5 days. *S. officinalis* (100 mg/kg) + Dox group (Sal + Dox): *S. officinalis* was given by gavage at a dose of 100 mg/kg/day for 5 days. A single dose of 30 mg/kg Dox was administered i.p. 30 min after the last administration. 24 hours after all applications, the rats were anesthetized with ketamine-xylazine (50 mg/kg-10 mg/kg), and the testicular tissues were carefully removed.

Histological analysis

The formalin-fixed testicular tissues were dehydrated with graded alcohols, then clarification with xylene and embedded in paraffin. 5 μm sections were taken and pasted onto slides. Paraffin was removed from tissue sections using xylene, and then a series of gradually decreasing alcohol concentrations were applied to immerse the sections in water. The sections were then stained with hematoxylin and eosin (H&E), dehydrated with a series of gradually increasing alcohol concentrations, cleared with xylene, and finally mounted with entellan[18]. Then, image areas belonging to the groups were photographed and analyzed using a light microscope (BX51; Olympus, Tokyo, Japan).

Evaluation of seminiferous tubule diameters

The measurement of seminiferous tubule diameters is used to examine the effects of new agents or treatments on testicular tissue. Changes in seminiferous tubule diameter help to evaluate the side effects or protective effects of treatment. In order to evaluate the seminiferous tubule diameters of different groups in H&E-stained sections, the averages of the measurements made in horizontal and vertical axes in 10 randomly selected microscopic fields were taken, and the obtained data were compared with statistical analysis[19].

Johnsen Testicular Biopsy Scoring (JTBS)

The testicular Johnsen testicular biopsy scoring (JTBS) is used to evaluate the efficiency of the spermatogenesis process and testicular functions. The histological structure of each seminiferous tubule is observed, and a score from 10 to 1 is given according to the presence or absence of the main cell types arranged in order of maturity [20]. In this study, a total of 20 tubule structures from 10 different areas randomly selected from each group were scored according to the scoring system.

Immunohistochemistry

The avidin-biotin-peroxidase method (Thermo Scientific, Waltham, MA) was used for immunohistochemical staining in order to ascertain

the immunoreactivity of the protein gene product (PGP) 9.5 and DAZL1 (germ cell marker) in testicular tissues. The tissues' 5 µm sections were deparaffinized and subsequently rehydrated by running them through a sequence of decreasing alcohols. To identify antigenic regions, sections were incubated in distilled water for five minutes at room temperature and then microwaved for twenty minutes in 0.01 M sodium citrate buffer. After washing with PBS, they were treated with 3% hydrogen peroxide for 10 minutes. After the washing step, diluted primary antibodies PGP 9.5 (Invitrogen, Lot: WL3461719E) and DAZL 1 (Elabscience, Lot: FC7343) were dropped and incubated at +4 °C overnight. After the washing process, biotinylated secondary antibodies and peroxidase-conjugated streptavidin were incubated for 10 minutes, respectively, and washing was performed in between. Sections were incubated with 3.3 'P-diaminobenzidine tetrahydrochloride. The sections were counterstained with hematoxylin, dehydrated through an ascending alcohol series, and cleared in xylene. Finally, they were mounted with entellan and examined under a microscope. Ten microscopic areas were randomly chosen from each preparation and photographed at 400x original magnification to determine the immunoreactivity density of the primary antibodies PGP 9.5 and DAZL 1 in testicular tissues. Image J software was used to compute the immunoreactivity by averaging the intensities[21].

Statistical analysis

The measurements of the diameter of the seminiferous tubules, the JTBS, and the immunoreactivity densities used in the study were taken. Each antibody's total immunoreactivity intensity was measured using the ImageJ software program. Images were taken at 400X magnification from 10 distinct microscopic fields for each group. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to determine whether the data was suitable for a normal distribution. Analysis was done using one-way analysis of variance (ANOVA) on normally distributed data. According to the results obtained with the GraphPad Prism 8.0.2. program, differences between groups were determined using the Tukey post hoc test for multiple comparisons. The significance limit of $p<0.05$ was accepted.

RESULTS

Under a light microscope, the general morphological appearance of the testis, seminiferous tubules, basement membrane, spermatogenic cells in the tubule epithelium, and interstitial areas was assessed using the H&E staining method.

The control group rats' testicular tissue was surrounded in a capsule made of tunica vasculosa and tunica albuginea. The seminiferous tubules had a rounded appearance, and the interstitial spaces were regular. The stratified epithelium lining the seminiferous tubules consisted of supporting Sertoli cells and spermatogenic cells. The nuclei of the spermatogonia were dark in appearance. The spermatogenic cells contained spermatogonia, primary spermatocytes with prominent large nuclei, secondary spermatocytes, spermatids, and spermatozoa arranged sequentially from the basal compartment to the adluminal compartment of the seminiferous tubule. When the testicular tissue sections belonging to the group to which Sal extract was applied were examined, it was observed that they exhibited a histological appearance similar to the control group with regular seminiferous tubule structures. Spermatogonia were generally located on the tubular basal lamina. A connective tissue similar to the control was also observed in the interstitial area. In the testicular tissues of the experimental groups induced with Dox**,** structural changes such as a significant decrease in tubular diameter, degeneration in seminiferous tubules, shedding of germ cells, and decreased spermatozoa in the tubular lumen were noted. In addition, there was hemorrhage in the interstitial connective tissue areas between the seminiferous tubules. In the testicular tissues of the treatment group where Sal and Dox were applied together when compared to the group where Dox was administered alone, it was noted that the damage in degenerated seminiferous tubules was significantly reduced, and an increase in seminiferous tubule epithelial cells was observed. In the Sal+Dox group, it was observed that the seminiferous tubule epithelium was arranged properly, and there were many spermatozoa in the lumen. Behind the Dox application, when the testicular tissues of the Salvia treatment group were examined, although not as much as the Dox group, the degenerated seminiferous tubules were noted. An increase in the seminiferous tubule epithelial cells of this group was observed compared to the damaged group, where Dox was administered alone. No hemorrhagic areas

were observed in either treatment group. It was observed that the histopathological change in the seminiferous tubules of the Dox+Sal group was significant compared to the Dox group but not as good as the Sal+Dox group (Figure 1) (H&E, X20, X40).

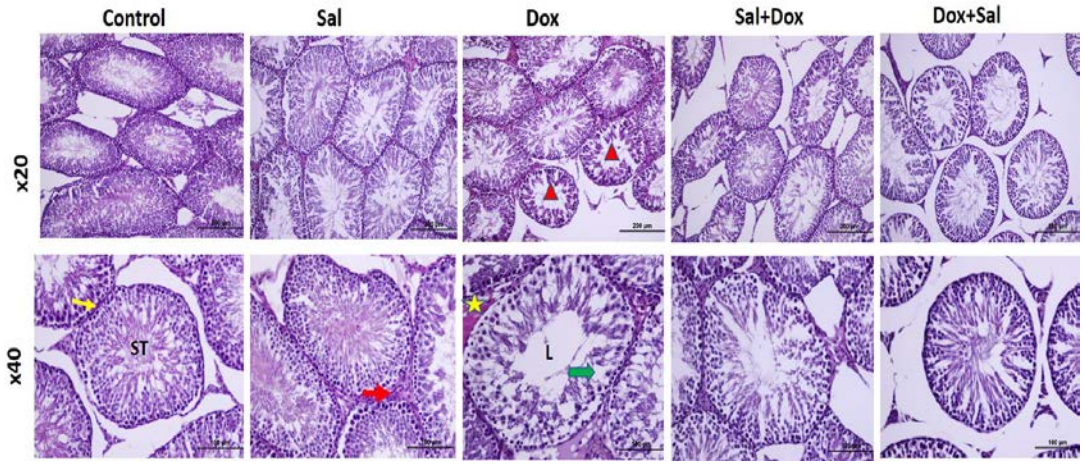

Figure 1. Microscopic images of testicular tissues of the groups with Hematoxylin-Eosin staining. ST: Seminiferous Tubule, L: Lumen, Yellow arrow: Basement membrane, Red arrow: Leydig cells, Yellow star: Hemorrhagic area, Red triangle: Degenerated seminiferous tubule, Green arrow: Germ cell shedding (20X, 40X).

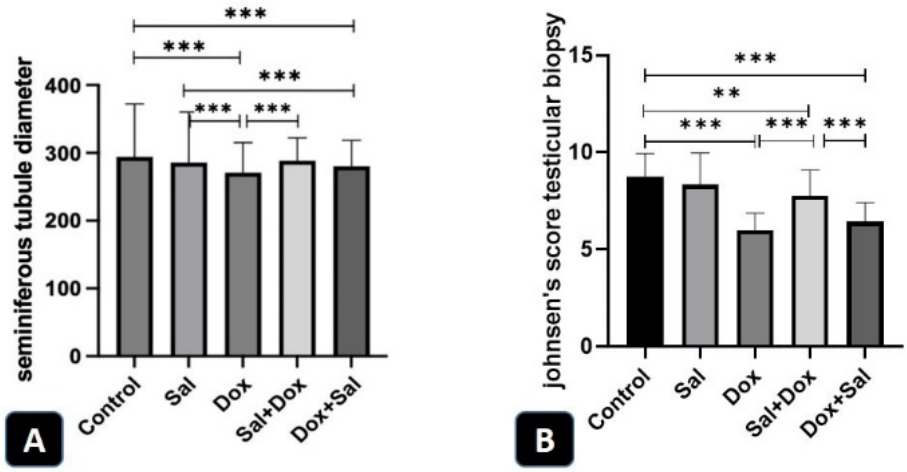

Figure 2. Statistical results of seminiferous tubule diameters (A) and JTBS (B) of the groups (p< 0.01, ***p < 0.001).**

Evaluation of seminiferous tubule diameters

Seminiferous tubule diameters are frequently used in histology and reproductive biology research to evaluate spermatogenesis and testicular health. Changes in tubule diameter may reveal how treatments affect testicular function. Seminiferous tubule diameters were higher in the Dox+Sal group than in the dox group, but the result was not

statistically significant ($p > 0.05$). When the groups were compared in terms of seminiferous tubule diameters, a significant increase was observed in the Sal+Dox group compared to the Dox group ($p < 0.001$) (Figure 2A). These findings suggest that Dox treatment directly compromises testicular tissue, while post-treatment *S. officinalis* exhibits a protective effect on the tissue to a certain extent, according to pre-treatment *S. officinalis*.

JTBS results

It is a widely used histopathological scoring system to evaluate the quality of spermatogenesis in testicular tissue. It is useful in evaluating testicular damage caused by diseases, treatments, or toxic exposures. The data obtained showed that there was a statistically significant decrease in the Dox group compared to the control group ($p < 0.001$). Additionally, there were fewer scores ($p<0.05$) in both Sal therapy groups than in the Dox group. Furthermore, after pre-incubation, *S. officinalis* was observed to be higher than in the Dox + Sal group (Figure 2B).Immunohistochemical analysis

PGP 9,5 Immunoreactivity

PGP 9.5 primary antibody expression in spermatogonia and Leydig cells was used to determine spermatogonial cells immunohistochemically. PGP 9.5 positive spermatogonia (brown color) and cytoplasmic staining were observed in the basement membrane of seminiferous tubules (Figure 3). When the immunoreactivity intensities were evaluated statistically for PGP 9.5, no significant difference was observed between the control group and the Sal group, while a statistically significant difference was observed between the other groups. There was a significant decrease in the Dox group compared to the control group ($p < 0.001$). A statistical significance was observed between the Dox group and both the Sal+Dox group and the Dox+Sal group ($p < 0.001$, $p < 0.01$, respectively) (Figure 4A).

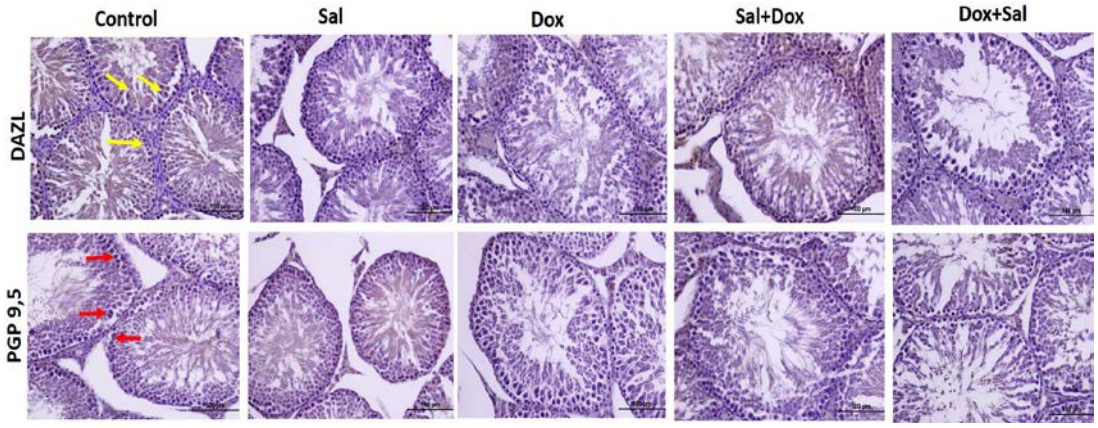

Figure 3. Microscopic image of DAZL1 and PGP 9.5 immunohistochemical staining in testicular tissues of groups (40X). Yellow arrow: DAZL1 positive cells, Red arrow: PGP 9,5 positive cells.

DAZL1 Immunoreactivity

DAZL1, specifically expressed in germ cells, staining was performed immunohistochemically to identify spermatogonial cells. Brown cytoplasm and nuclear staining were observed in spermatogonial cells (Figure 3). When the control group was compared with other groups, a significant difference was observed between the control group and both the Dox group and the Dox+Sal group ($p < 0.001$, $p < 0.01$, respectively). Significance was observed between the Sal group and the Dox+Sal group ($p < 0.01$). A significant difference was also observed between the Dox group and the Sal+Dox group ($p < 0.05$). The Shapiro-Wilk test and Kolmogorov-Smirnov test were used to determine whether the data in the groups showed normal distribution (Figure 4B).

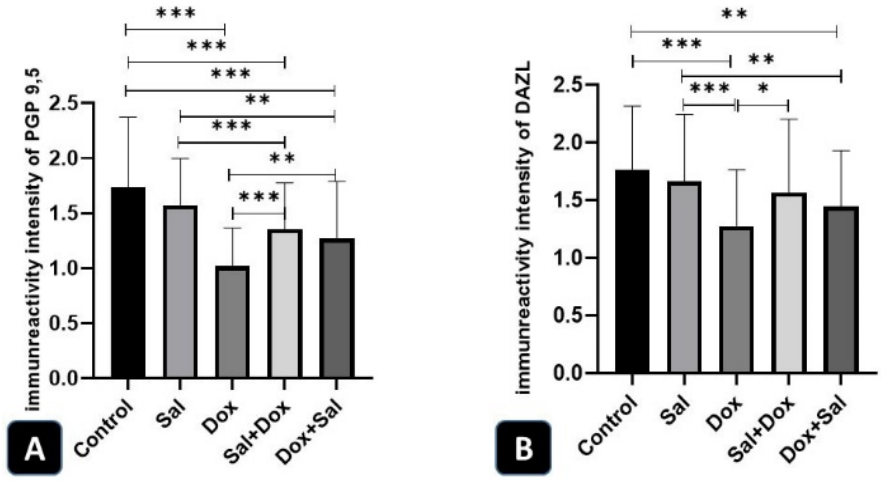

Figure 4. Statistical comparison of PGP 9.5 (A) and DAZL1 (B) immunoreactivity intensities between groups (*p < 0.05, **p < 0.01, ***p < 0.001).

DISCUSSION

Cancer is a complex disease that occurs as a result of damage to the genes that regulate cell growth and division. One of the current methods used in cancer treatment to destroy cancer cells is chemotherapy. It has been observed that the chemotherapeutic agents used to cause some negative effects on male reproductive functions[22]. Numerous chemotherapeutic agents can penetrate the blood-testis barrier and harm germ cells irreversibly. Due to their high mitotic activity, germinal epithelial cells are susceptible to chemotherapeutics. If stem cells are not harmed by chemotherapy, spermatogenesis might resume in a specific amount of time[13]. Bioactive phytochemicals and other natural compounds can lower the harmful effects of chemotherapy medications while also increasing their efficacy[23]. The purpose of this study was to use histopathological and immunohistochemical techniques to examine the potential preventive or therapeutic effects of *S. officinalis* hydromethanolic extract on Dox-induced testicular injury in male rats.

By attaching itself to tubulin, Docetaxel prevents normal microtubule depolymerization and disintegration, which results in cell cycle arrest and death. Docetaxel Dox is being used more and more in clinical settings to eradicate various cancer cell types due to its incredibly strong effects[24]. Most anticancer drugs, including docetaxel, face limitations in clinical use due to their extreme hydrophobicity, poor water solubility, low bioavailability, and high toxicity[25]. Additionally, docetaxel induces numerous adverse effects by promoting excessive reactive oxygen species production in certain tissues. Other known significant side effects, including neurotoxicity and liver toxicity, may lead to dose reduction or discontinuation of the drug[6]. It can also cause many adverse effects on healthy tissues[7,8].

The male reproductive organs known as the testes are essential for spermatogenesis and contain a variety of spermatogenic epithelial cells that are required for the formation of spermatozoa and the secretion of androgens. To comprehend the reproductive process and fertility, numerous research studies have focused on thoroughly analyzing the developmental process of the spermatogenic cells and histomorphological alterations in the testis[26]. It has been reported that after one week of exposure to chemotherapeutic agents such as cisplatin and doxorubicin, the number of seminiferous tubules is greatly reduced, and most importantly, there is a significant decrease in the number of proliferating SSCs[25]. Exposure to high concentrations of cisplatin continued its negative effects by reducing total germ cell numbers during both pre- and post-meiotic spermatogenesis[27]. It has been shown that docetaxel severely reduces sperm count, motility, and sperm abnormality in mice or rats and causes significant organ weight loss and histopathological atrophy in rat testes and

epididymis, while various Dox-loaded NPs reduce the side effects of Dox on the reproductive system[24]. In our study, in the testicular tissues of the Dox-induced experimental group, structural changes such as degeneration in seminiferous tubules, hemorrhage in the interstitial connective tissue areas between the seminiferous tubules, shedding of germ cells, and decreased spermatozoa in the tubular lumen were noted microscopically. In addition, when JTBS and seminiferous tubule diameter measurements were performed to examine the morphometric changes in the testicular tissue in the Dox group, a decrease was observed compared to the other groups.

Understanding the possibility of spermatogenesis recovery during chemotherapy is crucial; this recovery is contingent upon the survival or growth of spermatogonial stem cells. Accurately estimating reproductive risk and creating preventive measures depends on determining the detrimental effects of chemotherapy medications on testicular cells[28]. Therefore, the production of new drugs that will primarily affect cancer cells and will not harm normal cells is important.

S. officinalis is a very well-known and established medicinal and aromatic plant rich in various classes of phenolic compounds and terpenes. It has been used as a traditional medicine in many cultures and proved to have diverse pharmacological activities, including reproductive issues[11]. It has been shown that phytochemicals from *Salvia* species have cytotoxic effects on cancer cells with negligible harm to healthy cells[29]. In a mouse tumor model of Ehrlich ascites carcinoma, a hydroalcoholic extract of *S. officinalis* was reported to contribute to a reduction in tumor volume with fewer toxic effects when applied as an adjuvant with doxorubicin[30]. When we look at the literature, studies that used *S. officinalis* extract as a protective agent against the harmful effects of some toxic chemicals on testicular tissue have garnered attention. In rats given Sage water extract (SWE) (1 mL/kg b. wt) together with a hypercholesterolemic diet, it has been reported that SWE protects heart and testicular tissues by reducing the damaging effects caused by hypercholesterolemic diet due to its phenolic content and other antioxidant components[11]. Another study found that the aqueous extract of *S. officinalis* had a positive effect on several reproductive metrics and the pituitary-testicular hormone axis by increasing testosterone, LH, and FSH levels[31]. Seminiferous tubule widths, serum testosterone levels, and sperm numbers in male Wistar rats treated with 150 and 200 mg/kg S. officialis extract increased significantly[32]. *S. officinalis* extract has the ability to promote testicular growth as well as spermatozoa proliferation, maturation, and differentiation.

The presence of numerous bioactive components with high antioxidant activity, especially flavonoids and phenolic acid derivatives, was found to be responsible for the antioxidant activity of SWE[33]. In our study, it was observed that the seminiferous tubules in the testicular tissues of rats given Salvia extract (100 mg/kg) maintained their regular structure, the epithelial cells had a normal appearance, there was no change in the location of spermatogonial cells, and the tissue structure was generally preserved. It was found that administration of 100 and 200 mg/kg b.w. *S. officialis* hydroalcoholic extract to rats subjected to immobilization for 49 days improved the behavioral disorders of stressed rats and increased sperm parameters and reproductive capacity[34]. It was observed that histopathological lesions were observed in the kidneys, liver, and testes of mice treated with cyclophosphamide, but both doses of Salvia extract (0.3 and 0.6 g/kg b.w.) were not toxic and protected against cyclophosphamide-induced cytogenetic, biochemical, and pathological changes[35]. Co-administration of sage with uranyl acetate at doses of 190 mg/L and 380 mg/L counteracted uranyl acetate's toxic effects, leading to an improvement across all measured parameters, with the 380 mg/L sage dose showing more substantial effects[36]. The strong ability of sage to scavenge radicals, prevent lipid peroxidation, maintain endogenous antioxidant levels, and have anti-inflammatory and antioxidant effects may be related to the phytoactive compounds. In this study, unlike the literature, 70% methanolic leaf extract of *S. officinalis* (100 mg/kg) was applied to male rats in combination before or after a single dose of Dox (30 mg/kg, i.p.). When compared to the damaged group in which Dox was administered alone, a significant decrease in damage was noted in degenerated seminiferous tubules, and an increase in seminiferous tubule epithelial cells was noted. We found that the group that received Salvia after Dox had more epithelial cells and less seminiferous tubule degeneration. Histological analyses, however, indicated that the Sal+Dox group had a greater degree of recovery than this group.

The process by which spermatogonia in the testes develop into spermatozoa is known as spermatogenesis. The only adult cells in the seminiferous tubules that are necessary for

spermatogenesis and testis development are Sertoli cells (SCs). SCs and the substances they release are crucial elements of the testis microenvironment, promoting the development of germ cells into spermatozoa, spermatogonia, and markers (PGP9.5, DAZL1) associated with spermatogonial stem cells (SSCs)[37]. Furthermore, comprehending the course of spermatogenesis requires the identification, characterization, and monitoring of germ cell populations. Protein gene product 9.5 (PGP9.5) is a deubiquitinating enzyme that regulates mammalian gametogenesis and is a crucial regulator of germ cell death in the testes[38]. PGP9.5 is expressed in Leydig cells of stallions as well as in undifferentiated and early-developing spermatogonia. Spermatogenesis is explained in part by the expression and location of germ cells in SSCs. PGP9.5 controls mammalian gametogenesis and testicular germ cell apoptosis[39]. PGP9.5 expression was found in Leydig cells and spermatogonia[38]. In our study where we applied *S. officinalis* and Dox to male rats, both alone and in combination, it was observed that PGP9.5 expression in testicular tissue decreased with Dox application compared to control. However, when applied together before and after, it was seen that there was a significant increase compared to control and this increase was more effective in the Sal+Dox group compared to the other group.

The DAZL protein is important for mammalian spermatogenesis and has a critical role in the proliferation, development, and maturation of male germ cells[40]. It is known that DAZL1 protein, one of the SSC markers, is expressed in adult rat testis in a hormonally unchanged manner during meiosis. It is also stated that a decrease in DAZL1 may impair spermatogonial development and lead to a meiotic arrest in early pachytene[41]. It has been reported that amoxicillin and gentamicin cause severe oxidative stress in rat testes and negatively affect spermatogenesis by significantly reducing DAZL1 mRNA expression compared to the control group[42]. In our study, it was observed that DAZL1 expression decreased significantly in the Dox group compared to the control group. The decreased germinal epithelium may be related to the shedding of germ cells and the failure of basal germ cells to mature. When *S. officinalis* extract was administered together with Dox (Sal+Dox) as a protector, a significant increase closer to the control group was observed. According to the results of our study, it is thought that the protective effect of Salvia may be more effective than the therapeutic effect in testicular tissue. Considering the effect of DAZL1 on spermatogenesis and cell renewal, we believe that the significant increase in DAZL1 expression may be a physiological process in which the administered *S. officinalis* extract increases the expressions of PGP 9.5 and DAZL1 to protect the testicular tissue. The effects on testicular tissue were studied over a period of time in this study. Nonetheless, the limitation of our study was that the evaluation did not encompass assessing long-term effects or the potential for post-treatment reversibility. Despite a detailed examination of structural and immunohistochemical changes in testicular tissue, functional reproductive parameters such as sperm motility, concentration, and abnormalities remained outside the scope of the study.

In conclusion, studies on developing effective and reliable markers that can alleviate the negative effects of chemotherapeutic agents used in the treatment of testicular cancer on testicular tissue are of great importance. In this study, it was observed that *S. officinalis* hydromethanolic leaf extract has the potential to reduce the structural damage caused by Dox treatment in testicular tissue. The findings obtained may contribute to the development of treatment strategies for infertility by investigating the effects of individual anticancer agents on spermatogonial stem cells in more detail in future studies. In light of these limitations, planning more comprehensive studies in the future may enhance the clinical validity of the study.

Author Contributions: Concept/Design : MB, MO; Data acquisition: MO, DB; Data analysis and interpretation: MB, AY; Drafting manuscript: MO, MB; Critical revision of manuscript: MO, AY, MB; Final approval and accountability: MO, DB, AY, PG, MB; Technical or material support: MO, MB, PG; Supervision: MB; Securing funding (if available): n/a.
Ethical Approval: Approval was obtained from the Erciyes University Animal Experiments Local Ethics Committee (Decision No: 21/164).
Peer-review: Externally peer-reviewed.
Conflict of Interest: Regarding this article, the authors declare that there is no actual or potential conflict of interest.
Financial Disclosure: This study was supported by the Erciyes University Scientific Research Project Coordination Unit (Project no: EUBAP, TYL-2021-11448).
Acknowledgments: This study was presented at the 5th İnternational black Sea Modern Scientific Research Congress in Rize on 8-10 November 2023.

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