DOI: 10.37990/medr.1734022 Med Records 2026;1:1734022

MEDICAL RECORDS-International Medical Journal

Research Article

Protective Effects of Thymoquinone in Cisplatin-Induced Testicular Injury: A Focus on Cleaved Caspase-3 Expression

Nurcan Sahan[1], Damla Irmak[2], Kayihan Karacor[1]

[1]Department of Histology and Embryology, Faculty of Medicine, Duzce University, Duzce, Türkiye
[2]Department of Histology and Embryology, Sakarya Training and Research Hospital, Sakarya, Türkiye

Abstract

Aim: Cisplatin, an effective chemotherapeutic agent, has many side effects, including testicular damage that impairs spermatogenesis and may cause infertility. We aimed to eliminate or minimize the negative effects of cisplatin on testicular tissue with thymoquinone, which is increasingly investigated due to its biological properties.

Material and Methods: For this purpose, 29 Wistar albino rats were randomly divided into control (n = 4), sham (n = 4), cisplatin (n = 7), cisplatin + thymoquinone (n = 7), and thymoquinone (n = 7) groups. Corn oil was given to the sham group via gavage for 10 days. The cisplatin and cisplatin + thymoquinone groups received cisplatin (1.5 mg/kg/day, intraperitoneally) on days 3, 5, and 7, while thymoquinone (50 mg/kg/day, orally) was administered daily for 10 days to the cisplatin + thymoquinone and thymoquinone groups. On day 10, testicular tissues were removed under anesthesia and processed for histological and immunohistochemical analyses using cleaved caspase-3 antibody.

Results: A statistically significant decrease in cleaved caspase-3 expression, a key marker of apoptosis, was observed in the cisplatin + thymoquinone group compared to the cisplatin group (mean difference: −1.487, $p < 0.05$). It was determined that thymoquinone reduced testicular damage and germ cell apoptosis caused by cisplatin.

Conclusion: These results show that thymoquinone is effective in repairing the damage caused by cisplatin in testicular tissue and reducing spermatid apoptosis, but it needs to be supported by new studies to be used as a chemoprotectant.

Keywords: Antioxidant, Cisplatin, Cleaved Caspase-3, Thymoquinone, Apoptosis

INTRODUCTION

Chemotherapeutic drugs destroy tumor cells by stopping their growth and reproduction, but they can also damage healthy cells due to the similarities between tumor cells and healthy cells (1). Cisplatin is an antineoplastic agent used in the treatment of diverse solid tumors and hematological malignancies (2, 3). In addition to its serious side effects, cisplatin causes degeneration, necrosis, and interstitial edema in the testicular tissue (4) and a decrease in sperm concentration, a decrease in total normal sperm count, and a decrease in sperm motility in the testis (5, 6). Apoptosis is a type of programmed cell death distinguished by certain morphological and biochemical characteristics (7, 8). Apoptosis can be induced by multiple stimuli, such as oxidative stress and cytotoxic agents (9). Caspases are the principal proteases active during apoptosis (8, 9), and caspase-3 serves as a crucial executor in the apoptotic cascade (10). One of the best-known markers of apoptosis is the conversion of inactive procaspase-3 to its proteolytically active form, cleaved caspase-3 (11). Active caspase-3 causes DNA fragmentation and structural damage within the cell during apoptosis (12). In testicular tissue, apoptosis is crucial for homeostasis; however, excessive activation may result in the death of germ cells (13). Medicinal plants have long been recognized for their use in the treatment of many disorders, owing to their antioxidant

CITATION
Sahan N, Irmak D, Karacor K. Beyond the Curve: Protective Effects of Thymoquinone in Cisplatin-Induced Testicular Injury: A Focus on Cleaved Caspase-3 Expression. Med Rec 2026;1: 1734022. DOI: 10.37990/medr.1734022

Received: 18.07.2025 Accepted: 11.09.2025 Published: 01.03.2026
Corresponding Author: Assist. Prof. Dr. Kayihan KARACOR, Department of Histology and Embryology, Faculty of Medicine, Duzce University, 81620 Campus, Duzce, Türkiye
E-mail: dr.kayihan@hotmail.com

effects and broad therapeutic properties (14). Nigella sativa L., a member belonging to the Ranunculaceae family, is an annual flowering plant commonly referred to as black cumin (15, 16). It was stated that the essential oil obtained from the analysis of black cumin seed contained more than 60 compounds and that thymoquinone was the most abundant of these components (17). It has been reported that thymoquinone can also be used as a complement or alternative to chemotherapy treatment in cancer patients (18).

Recent studies have reported that thymoquinone modulates apoptosis in various cancer cell types through multiple pathways, including downregulation of Bcl-2, upregulation of Bax, activation of PPARγ, p53 signaling, caspase stimulation, and PARP cleavage (19-23). However, its potential effects on apoptosis in testicular tissue have not been fully elucidated.

Numerous studies have shown the role of reactive oxygen species (ROS) and oxidative stress in cisplatin-induced reproductive system toxicity (24). Thymoquinone, the principal component of black cumin seed oil, has demonstrated antioxidative, anti-inflammatory, and protective effects on the reproductive system (25). While the protective effects of thymoquinone on various organs have been previously studied, its specific role in preventing cisplatin-induced germ cell apoptosis through cleaved caspase-3 regulation in testicular tissue has not been fully elucidated. Moreover, existing studies often differ in terms of dosage, duration, and apoptotic markers used, making direct comparisons challenging. Our study contributes to the current literature by using a relatively higher thymoquinone dose (50 mg/kg) and focusing on early-stage apoptotic changes in spermatids via cleaved caspase-3 immunoreactivity. We hypothesized that thymoquinone would reduce cisplatin-induced germ cell apoptosis by suppressing cleaved caspase-3 expression.

MATERIAL AND METHODS

This study was conducted at the Duzce University Experimental Animals Application and Research Center with the approval of the Duzce University Experimental Animals Ethics Committee with the decision number 2018/5/4. This investigation was implemented in compliance with all institutional and international protocols for animal care and utilization. The guidelines for Animal Research: Reporting of In Vivo Experiments (ARRIVE) were adhered to appropriately. It was also supported by Duzce University Scientific Research Projects with the project number 2019. 04. 01. 904.

Twenty-nine Wistar albino rats, aged 2 to 3 months, were distributed at random among five groups: control (n=4), sham (n=4), cisplatin (n=7), cisplatin + thymoquinone (n=7), and thymoquinone (n=7). The experiment was completed with 29 Wistar albino male rats kept at 22-25ºC room temperature and a 12/12-hour light/dark cycle at optimal values under ventilation and satisfactory hygiene conditions, with free access to food and water. On the 3rd, 5th, and 7th days of the 10-day experiment, cisplatin was administered at a dose of 1.5 mg/kg/day i.p. (intraperitoneally), and thymoquinone was administered at a dose of 50 mg/kg/day via gavage. Corn oil, used as the vehicle for thymoquinone, was administered alone to the sham group; the control group received only saline. The administered cisplatin (26-28) and thymoquinone (29, 30) doses were selected based on literature studies.

At the end of ten days, the testicle tissue was carefully excised under anesthesia with 90 mg/kg ketamine and 10 mg/kg xylazine. The testicles were placed in Bouin solution for fixation. Thereafter, they were embedded in paraffin blocks of appropriate sizes, and 4-micron sections were taken with a microtome. The hematoxylin-eosin staining procedure was applied to the sections taken. For immunohistochemical examination, cleaved caspase 3 antibody (#9664, Cell Signaling Technology) was applied at a ratio of 1:100. The stained sections were covered with a coverslip by dropping Entellan. Then, the testicular tissue morphology of the groups was evaluated by examining them under an Olympus BX50 photomicroscope.

Semiquantitative evaluations

Cells were stained with cleaved caspase-3 antibody using immunohistochemical methods, and their staining intensities were evaluated semi-quantitatively (31, 32). Histopathological evaluation was performed in a blinded manner by two histologists who were experts in the field. In the evaluation, the scores were as follows: 0: negative, (+): weakly positive, +: positive, ++: intensely positive, and +++: very intensely positive (Table 1).

Statistical analysis

The GraphPad InStat (GraphPad Software Inc., San Diego, CA, USA) program was used for statistical evaluation of the immune reaction of animals in all groups. One-way ANOVA was used to evaluate general differences between groups; post-hoc analysis was performed with the Holm–Sidak multiple comparison test for parameters with significant differences. Additionally, mixed-effects model analysis was performed, considering the variability between rats. The significance threshold was accepted as $p < 0.05$.

RESULTS

Histological Examination

Testicular tissue sections from all experimental groups were stained with the hematoxylin-eosin method. To show the effects of thymoquinone against histopathological changes in the testis as a result of exposure to cisplatin, testicular tissue was evaluated according to shedding of germinal cells, separation in the seminiferous epithelium, and vacuolization criteria (33).

In the control group, germinal cells showing regular arrangement as an indicator of active spermatogenesis were identified in the testicular seminiferous tubule epithelium. According to the stages of spermatogenesis in the seminiferous tubule, the following were observed, respectively: Spermatogonia are located adjacent to the basal membrane, spermatids arise through mitotic and meiotic divisions, and mature spermatozoa are released into the lumen. In contrast, the cisplatin

Table 1: Semi-quantitative assessment of cleaved caspase-3 labeling in experimental groups.					
Cells / Groups	Control	Sham	Cisplatin	Cis + TQ	Thymoquinone
Spermatogonia	0	0	0	0	0
primer spermatocyte	0	0	+++	++	0
Spermatit	0	0	+	(+)	0
Sertoli cell	0	0	0	0	0
Leydig cell	0	0	+	(+)	0

group showed pronounced histopathological damage, including disorganized seminiferous tubules of varying diameters and shapes that were damaged in the testicles surrounded by a thin tunica albuginea. Vacuolization was observed in the walls of the damaged tubules, associated with cellular loss due to the dissociation of germinal cells. Testicular sections from the cisplatin + thymoquinone group demonstrated a marked improvement compared to the cisplatin group. It was observed that the gaps and separations in the seminiferous epithelium and the cellular debris in the lumen, which were seen due to germinal cell losses caused by separation and shedding in the seminiferous tubules, were reduced (Figure 1).

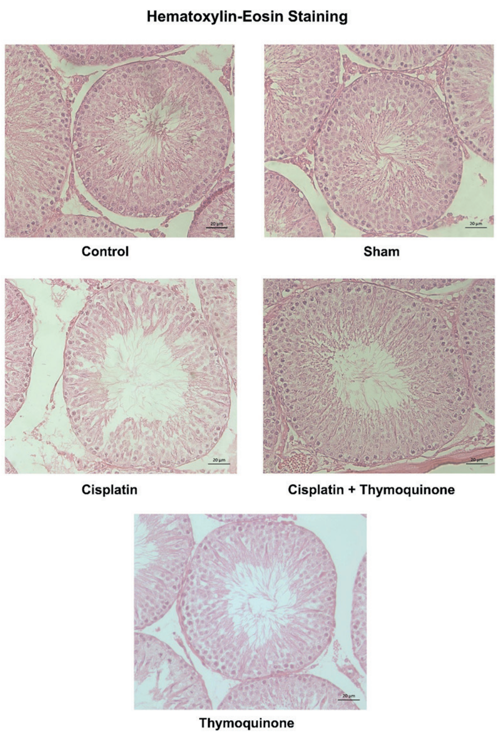

Figure 1. Hematoxylin-eosin staining in experimental groups.

Immunohistochemical Evaluation with Cleaved-Caspase 3 Antibody

A substantial difference was seen in the amount of cells positively stained with cleaved caspase-3 among the thymoquinone, cisplatin, and cisplatin + thymoquinone groups. Apoptosis was observed in spermatids in the cisplatin group. In the immunohistochemical staining section of the testicular tissue of rats in the cisplatin group with cleaved caspase-3, numerous apoptotic cell lines formed by spermatids were seen in the seminiferous tubule epithelium (Figure 2 and Table 1). In the immunohistochemical staining of the testicular tissue of the cisplatin + thymoquinone group with cleaved caspase 3, the staining intensity in spermatid cells was seen less (Figure 2 and Table 1). Compared to the cisplatin group, the number of cells stained with cleaved caspase-3 significantly decreased in the cisplatin + thymoquinone group. This result indicated that thymoquinone prevented apoptosis during cisplatin damage. In the immunohistochemical staining of the testicular tissue of rats in the thymoquinone group with cleaved caspase-3, it was seen that there were many mature sperm in the lumen of the seminiferous tubule with regular epithelium (Figure 2 and Table 1).

To detect the presence of apoptosis, the regions stained positively with cleaved caspase-3 were automatically calculated in the ImageJ program. Data were analyzed using GraphPad InStat software (GraphPad Software Inc., San Diego, CA, USA). Quantitative analysis of cleaved caspase-3 immunoreactivity revealed significant differences among the experimental groups. The mean intensity value of cleaved caspase-3 expression was highest in the cisplatin group (2.986 ± 0.6952), indicating elevated apoptosis levels compared to the control (0.8442 ± 0.1539), sham (0.8300 ± 0.1383), cisplatin + thymoquinone (1.499 ± 0.1423), and thymoquinone (0.7212 ± 0.1094) groups. Co-treatment with thymoquinone significantly reduced cleaved caspase-3 expression in the cisplatin + thymoquinone group compared to the cisplatin group ($p < 0.05$), suggesting a protective effect of thymoquinone against cisplatin-induced germ cell apoptosis. No significant difference was observed between the control and sham groups ($p = 0.815$), whereas the thymoquinone group showed a statistically significant difference compared to both ($p < 0.05$). Statistical analysis was performed using one-way ANOVA followed by Holm–Sidak's multiple comparisons test. Cleaved caspase-3 expression was significantly elevated in the cisplatin group compared to the other experimental groups ($p < 0.05$) (Figure 3).

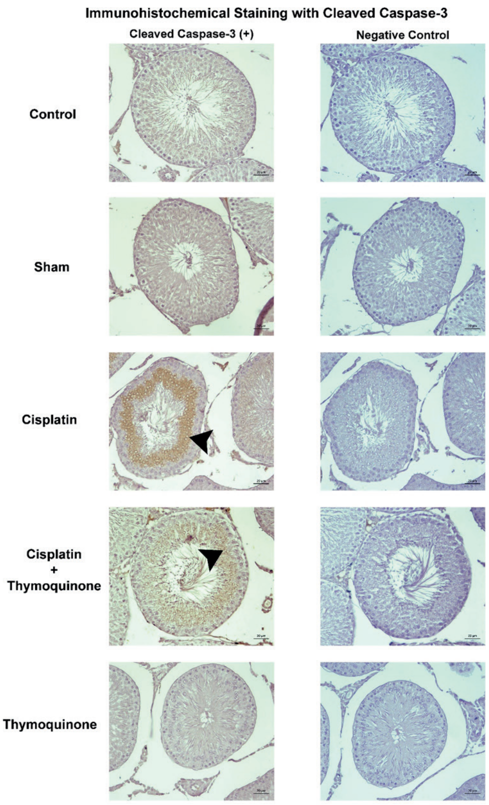

Figure 2. Immunohistochemical staining for cleaved caspase-3 in rat testicular tissue across experimental groups. Black arrowheads indicate cleaved caspase-3 expression in spermatids.

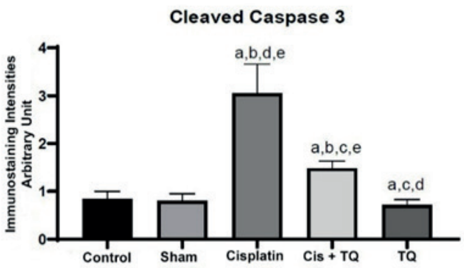

Figure 3. Analysis of cleaved caspase-3 immunostaining intensities in testicular tissue across experimental groups. Statistical significance was accepted at $p < 0.05$. a: compared to the control group, b: compared to the sham group, c: compared to the cisplatin group, d: compared to the Cis + TQ group, e: compared to the TQ group. (Cis: cisplatin; TQ: thymoquinone)

DISCUSSION

The findings of our investigation indicate that thymoquinone alleviates cisplatin-induced testicular injury by reducing apoptosis and preserving seminiferous tubule structure. Spermatogenic series cells are vulnerable to toxic agents, so genetic alterations are likely to occur in these cells. Leydig and Sertoli cells, which do not proliferate in adults, are the most resistant cells and continue to exist even after most cytotoxic drugs; however, these cells can suffer functional damage (34, 35).

Although cisplatin has been used successfully in cancer treatment for many years, studies on its mechanism of action are ongoing. It is thought that the drug's toxic effects occur as a result of binding to nuclear DNA, damaging transcription and DNA replication, and activating various signaling pathways. In addition, cisplatin disrupts mitochondrial integrity, decelerates the cell cycle, and then inhibits ATPase activity, altering cellular transport systems. Consequently, it leads to apoptosis, inflammation, necrosis, and ultimately cell death (36). Furthermore, beyond its DNA-damaging effects, cisplatin also induces the generation of reactive oxygen species (ROS), which further contribute to cell death by promoting oxidative stress (37).

The main effective basic compound found in the structure of Nigella sativa (black seed) essential oil is thymoquinone. Thymoquinone has been reported to protect multiple organs against oxidative damage and exhibits various pharmacological properties (18). Although there are numerous studies on the protective effects of thymoquinone against the harmful effects of cisplatin on other organs, studies evaluating its effects on testicular tissue remain limited.

Recent experimental evidence demonstrated that thymoquinone administration in a cisplatin-induced testicular damage model reduced oxidative stress by regulating apoptosis and autophagy processes (38). Thymoquinone has been shown to exhibit anti-apoptotic and antioxidant properties by regulating the TNF-α/OTULIN/NF-κB pathway in cisplatin-induced testicular damage. In contrast, our study focused on cleaved caspase-3 expression to assess apoptosis, and a lower cisplatin dose (1.5 mg/kg) and shorter experimental duration were used to detect early apoptotic changes, particularly in spermatids. The administration of a higher dose of thymoquinone (50 mg/kg vs 10 mg/kg in the referenced study) may have facilitated the apparent anti-apoptotic effect. The referenced study (39) complements our findings and contributes to the therapeutic effect of thymoquinone, combined with a different dosing and timing protocol in testicular injuries of various degrees. Furthermore, changes in autophagy markers, including reduced p62 and elevated LC3-II levels, were noted after thymoquinone treatment, signifying an enhancement in cellular homeostasis and tissue structure (40). These findings collectively indicate the possible use of thymoquinone as a chemoprotective drug in models of testicular toxicity.

Awadalla et al. (41) showed that Nigella sativa oil ameliorated cisplatin-induced oxidative and histological damage in testicular tissue, which is consistent with our findings and likely due to thymoquinone. Zhang et al. (42) investigated the time- and dose-dependent effects of cisplatin on testicular tissue by administering 1, 5, and 10 mg/kg to separate groups of mice and evaluating samples on days 1, 3, and 7. They reported a significant increase in germ cell apoptosis, particularly in the 10 mg/kg group on day 7, with apoptotic changes observed in spermatogonia, spermatocytes, and spermatids. Their findings indicate that cisplatin-induced germ cell death is contingent upon both dosage and duration. In our study, apoptotic cells were predominantly detected in spermatids. While previous studies have demonstrated that cisplatin-induced testicular damage leads to increased apoptosis across various germ cell stages and reduced sperm production, the absence of apparent cleaved caspase-3 immunolabeling in spermatogonia and primary spermatocytes in our findings may be attributed to the relatively low cisplatin dose (1.5 mg/kg) and the shorter duration of exposure. This interpretation aligns with the dose- and time-dependent increase in germ cell apoptosis reported by Zhang et al. (42).

There are also studies demonstrating thymoquinone's protective effect against the negative effects of cisplatin on organs other than the testicles (43-47). Although numerous studies support the protective effect of thymoquinone on testicular tissue, others report limited or no efficacy depending on experimental conditions. For example, 5 mg/kg thymoquinone administered to pregnant rats failed to prevent diclofenac-induced testicular cell loss in their offspring, with a significant reduction in Sertoli and Leydig cell numbers observed at postnatal week 4 (48). This result may be due to the relatively low dose of thymoquinone administered compared to our study (50 mg/kg). In the referenced study, testicular tissue was evaluated during the early postnatal period, when Sertoli and Leydig cells are still immature and therefore potentially more susceptible to toxic insults (49, 50), which could have contributed to the lack of protective effect observed.

While this study presents important findings, certain limitations should be considered. Apoptosis was assessed solely based on cleaved caspase-3 immunoreactivity. While this marker is widely used in literature and provides meaningful information, it may not be sufficient on its own to reflect the full complexity of the apoptosis process. Furthermore, the sample size may be relatively limited due to the ethical committees' framework. Due to financial constraints, confirmatory molecular methods could not be included in the study. These limitations are primarily due to ethical permission and resource constraints.

In this study, the light microscopic evaluation of testicular tissue in the cisplatin group revealed findings such as a decrease in the thickness of the seminiferous epithelium, degeneration of the tubular epithelium, shedding of spermatogenic cells, focal separation of the basal membrane, and interstitial disruption. Immunohistochemical analysis with cleaved caspase-3 antibody demonstrated an increased number of positively stained apoptotic cells in the cisplatin group. In contrast, this apoptotic response was markedly reduced in the cisplatin + thymoquinone group. These findings confirm that thymoquinone ameliorates cisplatin-induced testicular damage primarily by reducing apoptosis, as evidenced by decreased cleaved caspase-3 expression.

CONCLUSION

This study demonstrated that thymoquinone attenuates apoptosis in germ cells by reducing cisplatin-induced testicular damage and preserves seminiferous tubule integrity. The decrease in cleaved caspase-3 expression, particularly evident in spermatids, supports the apoptosis-protective effect of thymoquinone, thus suggesting that it may be a potential chemoprotective agent. However, further studies with larger sample sizes, at the molecular level, and additional multiple apoptosis markers are needed to confirm this effect and translate it into clinical practice.

Disclosure Statement

The authors declare no conflicts of interest.

Author contribution

Methodology: [NS, KK], Conceptualization: [NS, KK], Data curation: [NS, DI, KK], Formal analysis: [NS, KK], Investigation: [NS, KK], Validation: [NS, DI, KK], Visualization: [NS, DI, KK], Writing-Reviewing and Editing: [DI, KK], Supervision: [DI, KK].

Conflict of Interest

There is no potential conflict of interest relevant to this article.

Ethical approval

All experimental procedures were approved by the Duzce University Experimental Animals Ethics Committee (decision number: 2018/5/4).

REFERENCES

1. Mercantepe T, Unal D, Tumkaya L, Yazici ZA. Protective effects of amifostine, curcumin and caffeic acid phenethyl ester against cisplatin-induced testis tissue damage in rats. Exp Ther Med. 2018;15(4):3404-12.
2. Boulikas T, Vougiouka M. Cisplatin and platinum drugs at the molecular level (Review). Oncol Rep. 2003;10(6):1663-82.
3. Petrović M, Todorović D. Biochemical and molecular mechanisms of action of cisplatin in cancer cells. Facta Universitatis, Series: Medicine and Biology. 2016:12-8.
4. Hamza AA, Elwy HM, Badawi AM. Fenugreek seed extract attenuates cisplatin-induced testicular damage in Wistar rats. Andrologia. 2016;48(2):211-21.
5. Saral S, Ozcelik E, Cetin A, Saral O, Basak N, Aydin M, et al. Protective role of Diospyros lotus on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Andrologia. 2016;48(3):308-17.
6. Marcon L, Zhang X, Hales BF, Robaire B, Nagano MC. Effects of chemotherapeutic agents for testicular cancer on rat spermatogonial stem/progenitor cells. J Androl. 2011;32(4):432-43.
7. Öztürk F. Apopitoz. Journal of Turgut Ozal Medical Center. 2002;9(2).
8. M. E. Apopitosis. Arşiv. 2002;11.
9. Bassett EA, Wang W, Rastinejad F, El-Deiry WS. Structural and functional basis for therapeutic modulation of p53 signaling. Clin Cancer Res. 2008;14(20):6376-86.
10. O'Donovan N, Crown J, Stunell H, Hill AD, McDermott E, O'Higgins N, et al. Caspase 3 in breast cancer. Clinical Cancer Research. 2003;9(2):738-42.
11. Kamada S, Kikkawa U, Tsujimoto Y, Hunter T. Nuclear translocation of caspase-3 is dependent on its proteolytic activation and recognition of a substrate-like protein(s). J Biol Chem. 2005;280(2):857-60.

12. McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol. 2013;5(4):a008656.
13. Hikim AP, Wang C, Lue Y, Johnson L, Wang XH, Swerdloff RS. Spontaneous germ cell apoptosis in humans: evidence for ethnic differences in the susceptibility of germ cells to programmed cell death. J Clin Endocrinol Metab. 1998;83(1):152-6.
14. Al-Jassir MS. Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia. Food Chemistry. 1992;45(4):239-42.
15. Forouzanfar F, Bazzaz BS, Hosseinzadeh H. Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. Iran J Basic Med Sci. 2014;17(12):929-38.
16. Salama RH. Clinical and therapeutic trials of Nigella sativa. TAF Preventive Medicine Bulletin. 2010;9(5).
17. Dattner AM. From medical herbalism to phytotherapy in dermatology: back to the future. Dermatol Ther. 2003;16(2):106-13.
18. Khader M, Eckl PM. Thymoquinone: an emerging natural drug with a wide range of medical applications. Iran J Basic Med Sci. 2014;17(12):950-7.
19. Akgül A. Baharat Bilimi ve Teknolojisi, Gıda Teknolojisi Derneği Yayınları No: 15. Damla Matbaacılık ve Ticaret Konya. 1993:77-9.
20. Lei X, Lv X, Liu M, Yang Z, Ji M, Guo X, et al. Thymoquinone inhibits growth and augments 5-fluorouracil-induced apoptosis in gastric cancer cells both in vitro and in vivo. Biochem Biophys Res Commun. 2012;417(2):864-8.
21. Woo CC, Loo SY, Gee V, Yap CW, Sethi G, Kumar AP, et al. Anticancer activity of thymoquinone in breast cancer cells: possible involvement of PPAR-gamma pathway. Biochem Pharmacol. 2011;82(5):464-75.
22. Gali-Muhtasib H, El-Najjar N, Schneider-Stock R. The medicinal potential of black seed (Nigella sativa) and its components. Advances in Phytomedicine. 2006;2:133-53.
23. Das S, Dey KK, Dey G, Pal I, Majumder A, MaitiChoudhury S, et al. Antineoplastic and apoptotic potential of traditional medicines thymoquinone and diosgenin in squamous cell carcinoma. PLoS One. 2012;7(10):e46641.
24. Gevrek F, Erdemir F. Investigation of the effects of curcumin, vitamin E and their combination in cisplatin-induced testicular apoptosis using immunohistochemical technique. Turk J Urol. 2018;44(1):16-23.
25. El-Far AH. Thymoquinone Anticancer Discovery: Possible Mechanisms. Curr Drug Discov Technol. 2015;12(2):80-9.
26. Gürsoy A, Sade AG. Effects of diosmin administration on cisplatin-induced premature ovarian failure in a rat model. Journal of Contemporary Medicine. 2022;12(6):912-6.
27. Hamers FP, Brakkee JH, Cavalletti E, Tedeschi M, Marmonti L, Pezzoni G, et al. Reduced glutathione protects against cisplatin-induced neurotoxicity in rats. Cancer Res. 1993;53(3):544-9.
28. Perse M, Veceric-Haler Z. Cisplatin-Induced Rodent Model of Kidney Injury: Characteristics and Challenges. Biomed Res Int. 2018;2018:1462802.
29. Algaidi SA, Faddladdeen KA, Alrefaei GI, Qahl SH, Albadawi EA, HM AL, et al. Thymoquinone protects the testes of hypothyroid rats by suppressing pro-inflammatory cytokines and oxidative stress and promoting SIRT1 testicular expression. Front Pharmacol. 2022;13:1040857.
30. Alyoussef A, Al-Gayyar MM. Thymoquinone ameliorates testicular tissue inflammation induced by chronic administration of oral sodium nitrite. Andrologia. 2016;48(5):501-8.
31. Scudamore CL. Practical approaches to reviewing and recording pathology data. A practical guide to the histology of the mouse. 2014:25-41.
32. Meyerholz DK, Beck AP. Principles and approaches for reproducible scoring of tissue stains in research. Lab Invest. 2018;98(7):844-55.
33. Yaman Ö. Prepubertal sıçan testislerinde cisplatin maruziyetinin neden olduğu seminifer tübül hasarı ve erişkin sperm parametreleri üzerine L-Karnitinin koruyucu etkisi: Trakya Üniversitesi Sağlık Bilimleri Enstitüsü; 2015.
34. Guitton N, Brouazin-Jousseaume V, Dupaix A, Jegou B, Chenal C. Radiation effect on rat Sertoli cell function in vitro and in vivo. Int J Radiat Biol. 1999;75(3):327-33.
35. Miessler GL TD, İnorganik Kimya, 3. Baskı. , Palme Yayıncılık, Ankara,. Inorganic Chemistry. Third ed. Ankara: Palme Yayıncılık; 2009.
36. Solakoğlu Z. Apoptoz varlığı ya da yokluğu bir hastalık nedeni. Klinik gelişim. 2009;22(3):20-5.
37. Desoize B, Madoulet C. Particular aspects of platinum compounds used at present in cancer treatment. Critical reviews in oncology/hematology. 2002;42(3):317-25.
38. Shojaedini M, Hemadi M, Saki G, Fakhredini F, Khodayar MJ, Khorsandi L. Thymoquinone effects on autophagy, apoptosis, and oxidative stress in cisplatin-induced testicular damage in mice. J Assist Reprod Genet. 2024;41(7):1881-91.
39. Yalcin T, Kaya S, Yigin A, Agca CA, Ozdemir D, Kuloglu T, et al. The Effect of Thymoquinone on the TNF-alpha/OTULIN/NF-kappaB Axis Against Cisplatin-Induced Testicular Tissue Damage. Reprod Sci. 2024;31(8):2433-46.
40. Shojaedini M, Hemadi M, Saki G, Fakhredini F, Khodayar MJ, Khorsandi L. Thymoquinone Effects on the Expression of p62 Gene in Cisplatin-Induced Testicular Damage in Mice. Jentashapir Journal of Cellular and Molecular Biology. 2024;15(3).
41. Awadalla EA. Ameliorative effect of the crude oil of the Nigella sativa on oxidative stress induced in rat testes by cisplatin treatment. Biomedicine & Preventive Nutrition. 2012;2(4):265-8.
42. Zhang X, Yamamoto N, Soramoto S, Takenaka I. Cisplatin-induced germ cell apoptosis in mouse testes. Arch Androl. 2001;46(1):43-9.
43. Ahmed MA, El Morsy EM, Ahmed AA. Pomegranate extract protects against cerebral ischemia/reperfusion injury and preserves brain DNA integrity in rats. Life Sci. 2014;110(2):61-9.

44. Jakaria M, Cho DY, Ezazul Haque M, Karthivashan G, Kim IS, Ganesan P, et al. Neuropharmacological Potential and Delivery Prospects of Thymoquinone for Neurological Disorders. Oxid Med Cell Longev. 2018;2018:1209801.
45. Aslam H, Shahzad M, Shabbir A, Irshad S. Immunomodulatory effect of thymoquinone on atopic dermatitis. Mol Immunol. 2018;101:276-83.
46. Daba MH, Abdel-Rahman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol Lett. 1998;95(1):23-9.
47. Nagi MN, Mansour MA. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. Pharmacol Res. 2000;41(3):283-9.
48. Altindag F, Ragbetli MC. The effect of maternal treatment with diclofenac sodium and thymoquinone on testicular parameters in rat offspring. Rev Int Androl. 2021;19(1):34-40.
49. Sharpe RM, McKinnell C, Kivlin C, Fisher JS. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. Reproduction. 2003;125(6):769-84.
50. Franca LR, Hess RA, Dufour JM, Hofmann MC, Griswold MD. The Sertoli cell: one hundred fifty years of beauty and plasticity. Andrology. 2016;4(2):189-212.