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Erythropoietin Protective Role Against Methotrexate Testicular Adverse Effects

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Abstract

Background: The adverse effects of cytotoxic medications like methotrexate, particularly reproductive damage reported in the numerous experimental studies, limit their effectiveness as anticancer therapies. The current study's objectives were to identify potential histological and immune-histochemical unfavorable changes in the testicles due to methotrexate medication and to assess any possible protective effects of erythropoietin (EPO).

Methods and Results: The study included 60 mature male albino rats weighing 200-250 g. The animals were divided equally into three groups, each with 20 rats. In Group 1, the control group, the animals received intraperitoneal injections of normal saline twice a week for nine weeks at a dose of 0.5 mg/kg. For nine weeks, animals in Group 2 received intraperitoneal injections of methotrexate hydrate at 0.5 mg/kg twice a week. Animals in Group 3 received subcutaneous injections of 100 IU/kg recombinant human EPO once a week for nine weeks and intraperitoneal injections of methotrexate hydrate at a dosage of 0.5 mg/kg twice a week for nine weeks. Each rat was weighed every week throughout the examinations. An ELISA technique was used to measure the levels of testosterone, malondialdehyde, total antioxidant capacity, and ROS in blood serum. Morphological and histopathological changes in testicular tissue were assessed. The body weight in the rats treated with methotrexate was considerably lower than in control group rats. EPO showed clear androgenic and antioxidant activities and reduced the adverse effects of methotrexate on testicular histology. Our results suggest further research into the use of EPO as a drug to protect patients from the adverse effects of methotrexate.**(International Journal of Biomedicine. 2023;13(4):350-355.)**

Keywords: testicular tissue • erythropoietin • methotrexate • oxidative stress

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Abbreviations

EPO, erythropoietin; **MDA**, malondialdehyde; **ROS**, reactive oxygen species; **TAC**, total antioxidant capacity.

Introduction

One of the most dangerous side effects of chemotherapy is testicular destruction, which is commonly accompanied with oligozoospermia and azoospermia.(1) The antineoplastic medication methotrexate is frequently used to treat neoplasms. Many significant organs are affected by the side effects that patients who are receiving methotrexate medication experience. Numerous neoplastic diseases, such as acute lymphoblastic leukemia, non-Hodgkin's lymphoma, breast cancer, and testicular cancers, are treated with methotrexate.(2) Since methotrexate is an anti-metabolite medication, it is frequently

used to treat a variety of illnesses, including psoriasis and rheumatoid arthritis.[3] However, a significant danger is the unfavorable impact of methotrexate on rapidly growing organs. One of the methotrexate side effects is testicular damage. Oxidative stress, apoptotic alterations, inflammation, and changes in blood flow all play a role in how this effect manifests. [4] Testis and germ cell structure are harmed by oxidative stress. As a result, substances with antioxidant qualities may help shield testicular tissue from the adverse effects of oxidative stress from methotrexate. Erythropoietin (EPO), a glycoprotein hormone produced in the kidneys and secreted into the blood circulatory system, regulates erythropoiesis. EPO acts by binding to its cognate receptor (EPOR), which is expressed on the surface of erythroid progenitor cells. Signaling definitive erythrocyte progenitors via the EPOR leads to rescue from apoptosis, cell proliferation, expression of erythroid-specific proteins such as hemoglobin, and ultimately terminal differentiation into mature, enucleated definitive erythrocyte.[5] EPO is induced by hypoxia via the hypoxia-inducible factor family of transcription factors. Recently, it has been shown that EPO possesses cytoprotective properties in addition to its typical hematopoietic function.[6] As a growth factor, EPO is essential for cell development and neovascularization.

Our team previously investigated and evaluated the preventive effects of EPO and sildenafil intraperitoneally injected into adult rats who underwent testicular torsion and detorsion. The research showed that EPO treatment had milder histological changes than the control group, with sildenafil likely having an improved action.[7] Numerous studies have described the cytoprotective properties of EPO in various organs, including the brain, kidney, heart, and retina, and have suggested that EPO may have therapeutic value. However, little evidence is presented regarding the testicles. [8] The current study's objectives were to identify potential histological and immune-histochemical unfavorable changes in the testicles due to methotrexate medication and to assess any possible protective effects of EPO.

Materials and Methods

Our work was conducted in accordance with the PSA University's Al-Kharj Ethical Committee's guidelines for the use and care of animals in research (SCBR-136-2022). The protocol for this study was also created in compliance with the ethical standards of the Laboratory of the International Committees for the Protection of Animal Rights.

The study included 60 mature male albino rats weighing 200-250 g. Rats were kept in the vivarium at a temperature of 21–22°C with a 12-hour light/dark cycle, fed a conventional rat diet, and allowed unlimited access to water.

The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care, following the protocol approved by the Institutional Animal Care and Use Committee of the PSAU (SCBR-136-2022).

We purchased methotrexate tablets (2.5 mg) from the Orion Corporation (Espoo, Finland). EPO (5000 IU) was purchased from Recormon (Roche Diagnostics GmbH, Mannheim, Germany).

Following two weeks of acclimation, the animals were divided equally into three groups, each with 20 rats. In Group 1, the control group, the animals received intraperitoneal injections of normal saline twice a week for nine weeks at a dose of 0.5 mg/kg. For nine weeks, animals in Group 2 received intraperitoneal injections of methotrexate hydrate at 0.5 mg/kg twice a week. Animals in Group 3 received subcutaneous injections of 100 IU/kg recombinant human EPO once a week for nine weeks and intraperitoneal injections of methotrexate hydrate at a dosage of 0.5 mg/kg twice a week for nine weeks.

Each rat was weighed every week throughout the examinations. Blood samples were drawn from the retro-orbital venous plexus using a capillary tube. The serum was separated by centrifugation and stored at -20°C. An ELISA technique was used to measure the levels of testosterone,[9] malondialdehyde (MDA), total antioxidant capacity (TAC), and ROS in blood serum. Then, ether inhalation anesthesia was given to the rats. The testicles were removed from the rats (Figure 1) at the designated dates via a median abdominal incision, stored, and then processed for paraffin slices. All specimens were kept in a 10% formol saline solution for three days to prepare them for paraffin sectioning. The testes were immediately removed and cured in 10% formal saline solution for three days. After a day of treatment with 70%, 90%, and finally absolute alcohol, the specimens were totally dehydrated and then subjected to three hours of treatment with ethyl alcohol at increasing concentrations. For a full day, the samples were benzene-cleared. The cleaned samples were submerged in paraffin wax three times for one hour each. After that, the samples were firmly embedded in paraffin wax. For general morphological analysis and the detection of histopathological changes, thin paraffin sections (3–4 microns) were prepared, mounted on charged glass slides, and stained with H&E. Mallory's trichrome stain was used to determine the different groups' tubular diameters and glycogen levels in the seminipherous tubules. In addition, the average number of seminiferous tubular germinal cells expressing caspase-3 in the study's various groups, as determined by sections stained with caspase-3, were studied.[10]

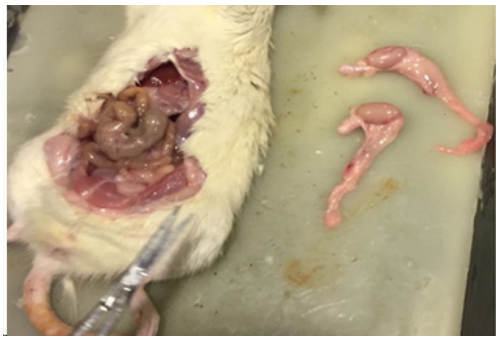

Fig. 1. *The removed testicles.*

Statistical analysis was performed using statistical software package SPSS version 17.0 (Chicago: SPSS Inc.). Baseline characteristics were summarized as frequencies and

percentages for categorical variables and as mean±SD for continuous variables. Multiple comparisons were performed with one-way ANOVA and Tukey HSD post-hoc test. A probability value of $P<0.05$ was considered statistically significant.

Results

The body weight in Group 2 was considerably lower than in Group 1 (Table 1). Compared to the control group, the TAC was not significantly altered by methotrexate treatment in Group 2. However, EPO administration in Group 3 raised antioxidant levels, compared to Group 1. Group 3 showed a significant increase in testosterone level and ROS compared to Group 2. Malondialdehyde levels in Group 2 did not differ from those of Group 1 (Table 2).

Table 1.

The body weight in the study groups.

Parameters	Group 1	Group 2	Group 3
Weight at the start of the experiment (g)	280 ± 4.33	231 ± 3.43	260 ± 5.22
Weight at the end of the experiment (g)	290 ± 3.11	210 ± 2.44	270 ± 5.31
Percentage (%)	+3.44 %	-9.09%	+ 3.7%

Table 2.

Effects of EPO and methotrexate treatment on the levels of testosterone, malondialdehyde, and ROS in serum.

	Group 1 (1)	Group 2 (2)	Group 3 (3)	Statistic
Testosterone, ng/ml	8.3±1.19	7.26±1.88	9.99±3.18	F=7.562, P= 0.0012 P_{1-2}=0.3139 P_{1-3}=0.0526 P_{2-3}=0.0009
TAC, mmol/g	5.77±2.99	5.11±2.23	8.77±3.98	F=7.674, P= 0.0011 P_{1-2}=0.7859 P_{1-3}=0.0106 P_{2-3}=0.0015
ROS, $\mu mol \cdot min^{-1}$	4.32±0.77	1.51±0.31	6.12±1.33	F=131.722, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{2-3}=0.0000
MDA, nmol/l	2.39±0.56	2.11±0.44	2.94±1.22	F=5.361, P= 0.0074 P_{1-2}=0.5268 P_{1-3}=0.0923 P_{2-3}=0.0059

Sections from the control group displayed normal seminiferous tubule histology, an entire luminal spermatogenic series, and interstitial Leydig cells. Spermatogenic cells in various developmental stages and mature sperms were found in the lumen of the seminiferous tubules. Two cell types were present in seminiferous tubules: spermatogenic and Sertoli cells (Figure 2). Spermatogonia, spermatocytes, spermatids, and spermatozoa were placed in that order from the basal to the adluminal compartments. Fibroblasts, an average quantity of collagen fibers, blood vessels, and Leydig cells were present in the interstitial space, although the space was small. Primary spermatocytes were located near spermatogonia and had big, rounded central nuclei, whereas spermatogonia were basal locations and had small, spherical nuclei. Primary spermatocytes and spermatids were found nearby. Their elongated, strongly pigmented nuclei allowed for the identification of the elongated spermatids. Spermatogonia sitting on the basement membrane were found to be interspersed with Sertoli cells with oval nuclei (Figure 2). Polygonal or spherical Leydig cells with single or double nuclei and granular cytoplasm surrounding the blood arteries in the interstitial tissue were determined. These cells could be seen alone or in groups (Figure 2).

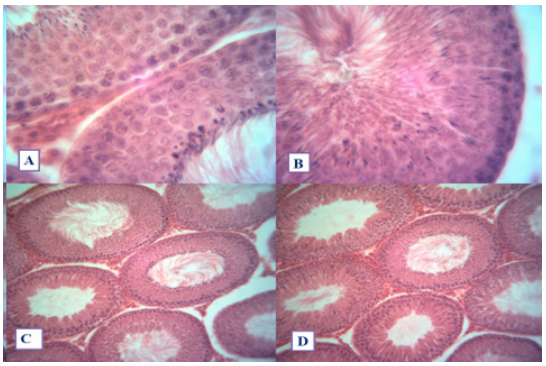

Fig. 2. *A normal connection of the germ cells and normal architecture of the interstitial tissue in a section of rat testicular tissue stained with H&E. Control group. A & B - 400x magnification; C and D - 200x magnification.*

Methotrexate caused testicular injury as evidenced by histopathologic examination, which showed seminiferous tubule degeneration as manifested by sloughing, atrophy, and germ-cell degeneration. The tubules' lining was disordered; most had a few layers of spermatogonia or a few layers of spermatocytes. The diameter of the tubules decreased. The uneven basal lamina surrounding each tubule reduced the height of the layers. The interstitial area was enlarged (Figure 3).

Numerous deteriorated eosinophilic cells with condensed chromatin mixed with apoptotic bodies and cell debris were seen in addition to vacuolar degeneration. Seminiferous tubule morphologies in the testicular tissue of the rats treated with methotrexate were irregular, and their diameters were significantly smaller than those of the control group (Figures 3 and 4). Compared to the rats treated with methotrexate alone, those treated with EPO significantly recovered the seminiferous tubule diameters. Additionally, the germinal epithelium had blatant disarray along with aberrant cellular attachment (Figure 4, Table 3). Most of the cells observed were spermatogonia cells. Multinucleated cells with two or three nuclei were also found in interstitial tissue. The interstitial connective tissue included an amorphous substance, which caused the connective tissues to break down noticeably and enlarge the interstitial tissue spaces (Figure 4).

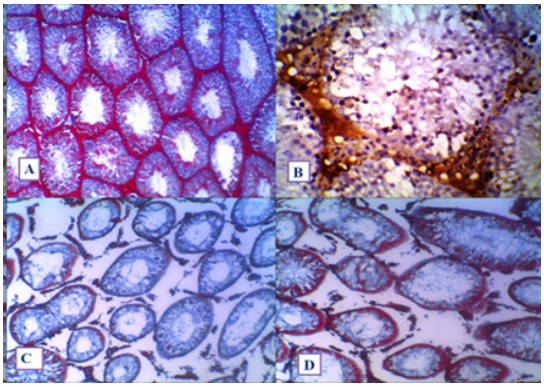

Fig. 3. *A: Collagen fibers are distributed normally in the interstitial tissue surrounding the seminiferous tubules in testicular tissue from the control group. Mallory's trichrome (200x magnification). B: Immunostaining for caspase-3 reveals a minimal expression in the seminiferous tubules' germinal cells. Control group (400x magnification). C and D: Methotrexate-treated tissue reveals a significant decrease in collagen fibers in the interstitial tissue surrounding the seminiferous tubules. Mallory's trichrome (200x magnification).*

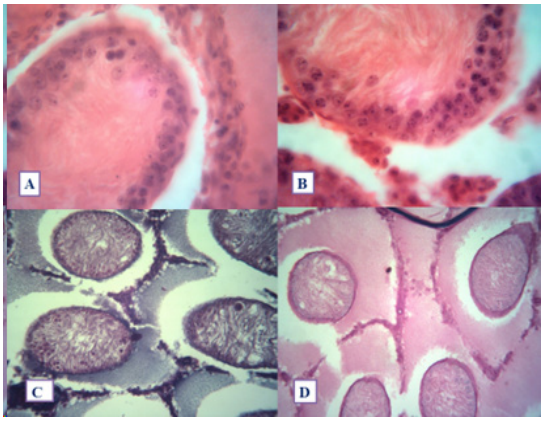

Fig. 4. *A and B: The germinal epithelium in the methotrexate-treated rat's H&E-stained piece is disorganized and uneven (400x magnification). C and D: Testicular tissue from a rat treated with methotrexate shows increased diameter of the interstitial spaces. (200x magnification). C) Mallory's trichrome (200x magnification). D) H&E staining (200x magnification)*

Compared to Group 2, Group3 displayed a large increase in collagen fibers and a significant recovery of the interstitial tissue to normal levels. Seminiferous tubules had a better organizational structure and were covered in conventional basement membrane. There were more spermatozoa tails in the lumina, fewer tubules had shed the germinal epithelium from their basement membrane, and the interstitial space was less than in Group 2. Better structured germinal epithelium-lined seminiferous tubules were found. The basement membrane supported the Sertoli cell. Wide intercellular gaps might be seen in some locations. There were not many Leydig cells with vesicular nuclei in the interstitial tissue (Figure 5).

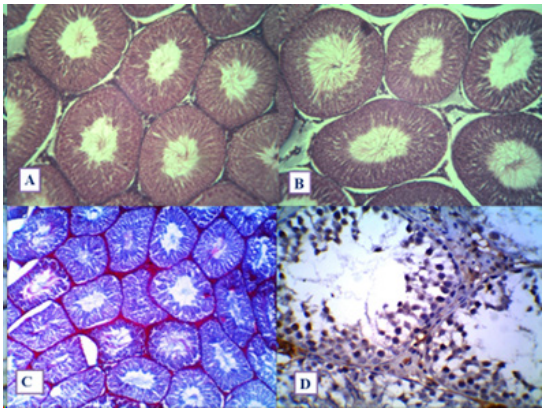

Fig. 5. *A and B: Testicular tissue from the EPO-treated rat has partial recovery to normal structure (H&E, 200x magnification); C: In comparison to the control group, testicular tissue treated with EPO exhibits a more or less identical distribution of collagen fibers in the tissue surrounding the seminiferous tubules. Mallory's trichrome (200x magnification). D: Caspase-3 immunostaining in germinal cells is moderately expressed in a region that has received erythropoietin treatment (400x magnification).*

The seminiferous tubules germinal epithelium was significantly reduced, underwent apoptotic alterations, was disorganized, and was depleted in Group 2, compared to the control group. Compared to Group 2, there was a markedly substantial recovery of the germinal epithelium in Group 3.

Table 3.

Histopathological changes in the testicles after nine weeks treatment in the study groups.

	Diameter of seminiferous tubules (μm)	Thickness of seminiferous tubules (μm)	Number of caspase-3+ cells (in 100 tubules	Area percentage of the collagen fibers
Group 1	179. 22 ± 14.11	100.76 ± 8.66	11.99 ± 2.11	139.11 ± 19.98
Group 2	132.57 ± 36.22	191.70 ± 31.49	38.55 ± 2.96	70.23 ± 17.76
Group 3	189.89 ± 11.87	89.42 ± 16.33	14.99 ± 3.99	151.89 ± 18.89
Statistic	F=33.753, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.3285 P_{2-3}=0.0000	F=141.454, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.2136 P_{2-3}=0.0000	F=435.751, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.0097 P_{2-3}=0.0343	F=108.042, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.0911 P_{2-3}=0.0000

The testicular tissue from the control testes had modest caspase-3 immunostaining and a small number of caspase-3-positive cells in the seminiferous tubules. Compared to the control group, the number of caspase-3-positive cells in the seminiferous tubules increased significantly in Group 2. Additionally, Group 3 showed a markedly significant increase in caspase-3-positive cells in the seminiferous tubules compared to the control group (Figure 5, Table 3).

Discussion

Our study showed that rats treated with methotrexate had testicular damage, which was demonstrated biochemically by decreased testosterone levels as well as several histological alterations. The adverse effects of cytotoxic medications like methotrexate, particularly reproductive damage reported in the numerous experimental studies, limit their effectiveness as anticancer therapies.[11] According to data from earlier investigations, there is sperm DNA damage, sperm count decline, and seminiferous tubule disorder.[12] Methotrexate toxicity causes cellular macromolecule damage by accumulating ROS and depleting antioxidants.[13] Various therapeutic agents have been tested for their ability to reduce testicular damage during chemotherapy. In animals, many tissues and organs, including the spinal cord, kidneys, liver, heart, lungs, brain, intestines, and retina, have been shown to suffer less ischemia damage when given EPO and sildenafil.[14]

Methotrexate has been investigated in several animal models, and dose, time, and animal species influenced drug toxicity. Previous studies have shown that exposure of male mice, like many other animals, to methotrexate results in similar adverse effects in the testes, including loss of testicular, seminal vesicle, and prostate weight, as well as morphological abnormalities of the testes.[15] In the methotrexate group without EPO, we observed degeneration of the seminiferous tubules, a significant decrease in spermatogenic cells, pyknotic nuclei, and cytoplasm vacuolization. These results may be because dihydrofolate reductase, an essential enzyme required for healthy DNA synthesis, was inhibited, preventing primary spermatocytes and spermatids from successfully replicating their DNA.[16] In our study, methotrexate caused seminiferous tubule degeneration, a significant drop in spermatogenic cells, pyknotic nuclei, and vacuolated cytoplasm. According to a recent study, Sertoli cells regulate the spermatogenic process; therefore, cytoplasmic vacuolation may be caused by damage to Sertoli cells.[17] In a study by Yucel et al.,[18] methotrexate administration increased histopathological damage, TAC (total antioxidant capacity), TOS (total oxidative status), and OSI (oxidative stress index) levels in hepatic tissue. Numerous studies have shown that EPO has cytoprotective properties in organs such as the brain, kidneys, heart, and retina.[19] Infusion of recombinant human EPO in a rat model of ischemic brain injury reduced neuronal death by reducing the levels of pro-inflammatory cytokines such as TNF-alpha, IL-6, and MCP-1.[20]

In conclusion, the present study shows that EPO has clear androgenic and antioxidant activities and reduces the adverse effects of methotrexate on testicular histology. These results also suggest further research into the use of EPO as a drug to protect patients from the adverse effects of chemotherapy.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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References

1. Aboul Fotouh GI, Abdel-Dayem MM, Ismail DI, Mohamed HH. Histological Study on the Protective Effect of Endogenous Stem Cell Mobilization in Busulfan-Induced Testicular Injury in Albino Rats. J Microsc Ultrastruct. 2018 Oct-Dec;6(4):197-204. doi: 10.4103/JMAU.JMAU_35_18.
2. Jensen SB, Mouridsen HT, Reibel J, Brünner N, Nauntofte B. Adjuvant chemotherapy in breast cancer patients induces temporary salivary gland hypofunction. Oral Oncol. 2008 Feb;44(2):162-73. doi: 10.1016/j.oraloncology.2007.01.015.
3. Dadhania VP, Tripathi DN, Vikram A, Ramarao P, Jena GB. Intervention of alpha-lipoic acid ameliorates methotrexate-induced oxidative stress and genotoxicity: A study in rat intestine. Chem Biol Interact. 2010 Jan 5;183(1):85-97. doi: 10.1016/j.cbi.2009.10.020.
4. Abdelzaher WY, Khalaf HM, El-Hussieny M, Bayoumi A, Shehata S, Refaie M. Role of nitric oxide donor in methotrexate-induced testicular injury via modulation of pro-inflammatory mediators, eNOS and P-glycoprotein. Hum Exp Toxicol. 2020 Dec;39(12):1700-1709. doi: 10.1177/0960327120940361.
5. Kimura T, Sonoda Y, Iwai N, Satoh M, Yamaguchi-Tsukio M, Izui T, Suda M, Sasaki K, Nakano T. Proliferation and cell death of embryonic primitive erythrocytes. Exp Hematol. 2000 Jun;28(6):635-41. doi: 10.1016/s0301-472x(00)00156-9.
6. Kahraman H, Kurutaş E, Tokur M, Bozkurt S, Cıralık H, Kabakcı B, Köksal N, Balkan V. Protective effects of erythropoietin and N-acetylcysteine on methotrexate-induced lung injury in rats. Balkan Med J. 2013 Mar;30(1):99-104. doi: 10.5152/balkanmedj.2012.078.
7. Zavras N, Kostakis ID, Sakellariou S, Damaskos C, Roupakas E, Tsagkari E, Spartalis E, Velaoras K, Dontas IA, Karatzas T. Comparison of erythropoietin and sildenafil protective role against ischemia/reperfusion injury of the testis in adult rats. Int Urol Nephrol. 2014 Apr;46(4):731-6. doi: 10.1007/s11255-013-0569-x.
8. Haine L, Yegen CH, Marchant D, Richalet JP, Boncoeur E, Voituron N. Cytoprotective effects of erythropoietin: What about the lung? Biomed Pharmacother. 2021 Jul;139:111547. doi: 10.1016/j.biopha.2021.111547.

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9. Maruyama Y, Aoki N, Suzuki Y, Ohno Y, Imamura M, Saika T, Sinohara H, Yamamoto T. Sex-steroid-binding plasma protein (SBP), testosterone, oestradiol and dehydroepiandrosterone (DHEA) in prepuberty and puberty. Acta Endocrinol (Copenh). 1987 Jan;114(1):60-7. doi: 10.1530/acta.0.1140060.
10. Pallet N, Bouvier N, Legendre C, Beaune P, Thervet E, Choukroun G, Martinez F. Antiapoptotic properties of recombinant human erythropoietin protects against tubular cyclosporine toxicity. Pharmacol Res. 2010 Jan;61(1):71-5. doi: 10.1016/j.phrs.2009.08.010.
11. Ilbey YO, Ozbek E, Simsek A, Otunctemur A, Cekmen M, Somay A. Potential chemoprotective effect of melatonin in cyclophosphamide- and cisplatin-induced testicular damage in rats. Fertil Steril. 2009 Sep;92(3):1124-1132. doi: 10.1016/j.fertnstert.2008.07.1758
12. Pınar N, Çakırca G, Özgür T, Kaplan M. The protective effects of alpha lipoic acid on methotrexate induced testis injury in rats. Biomed Pharmacother. 2018 Jan;97:1486-1492. doi: 10.1016/j.biopha.2017.11.078.
13. Rofaeil RR, Ibrahim MA, Mohyeldin RH, El-Tahawy NF, Abdelzaher WY. Role of EGF/ERK1/2/HO-1 axis in mediating methotrexate induced testicular damage in rats and the ameliorative effect of xanthine oxidase inhibitors. Immunopharmacol Immunotoxicol. 2023 Mar 8:1-10. doi: 10.1080/08923973.2023.2181684.
14. Kostakis ID, Zavras N, Damaskos C, Sakellariou S, Korkolopoulou P, Misiakos EP, Tsaparas P, Vaos G, Karatzas T. Erythropoietin and sildenafil protect against ischemia/reperfusion injury following testicular torsion in adult rats. Exp Ther Med. 2017 Jun;13(6):3341-3347. doi: 10.3892/etm.2017.4441
15. Yu L. Free radical scavenging properties of conjugated linoleic acids. J Agric Food Chem. 2001 Jul;49(7):3452-6. doi: 10.1021/jf010172v.
16. Shrestha S, Dhungel S, Saxena AK, Bhattacharya S, Maskey D. Effect of methotrexate (MTX) administration on spermatogenesis: an experimental on animal model. Nepal Med Coll J. 2007 Dec;9(4):230-3.
17. Abdul-Hamid M, Abdel-Reheim ES, Hegazy W, Allam AA, Othman SI, ALqhtani H, Abdel-Kawi SH. Impact of gervital against histopathological, ultrastructural, and biochemical alterations caused by methotrexate or azathioprine in albino rat testis. Environ Sci Pollut Res Int. 2023 Feb;30(8):21914-21926. doi: 10.1007/s11356-022-23588-3.
18. Yucel Y, Oguz E, Kocarslan S, Tatli F, Gozeneli O, Seker A, Sezen H, Buyukaslan H, Aktumen A, Ozgonul A, Uzunkoy A, Aksoy N. The effects of lycopene on methotrexate-induced liver injury in rats. Bratisl Lek Listy. 2017;118(4):212-216. doi: 10.4149/BLL_2017_042.
19. Li GQ, Chen M. Cardioprotective effect of erythropoietin in rats with acute myocardial infarction through JNK pathway. Eur Rev Med Pharmacol Sci. 2019 Jan;23(3 Suppl):153-160. doi: 10.26355/eurrev_201901_18642.
20. Villa P, Bigini P, Mennini T, Agnello D, Laragione T, Cagnotto A, Viviani B, Marinovich M, Cerami A, Coleman TR, Brines M, Ghezzi P. Erythropoietin selectively attenuates cytokine production and inflammation in cerebral ischemia by targeting neuronal apoptosis. J Exp Med. 2003 Sep 15;198(6):971-5. doi: 10.1084/jem.20021067.