Iraqi Journal of Veterinary Sciences, Vol. 37, Supplement IV, 2023 (211-217)
Proceedings of the 4th Scientific Conference, College of Veterinary Medicine, University of Karbala

Iraqi Journal of Veterinary Sciences

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Effects of methotrexate on hepatic and testicular tissues in male rabbits: Histological, hormonal and biochemical analysis

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Article information

Article history:
Received 03 March, 2023
Accepted 22 November, 2023
Available online 02 January, 2024

Keywords:
Chemotherapy
Hepatotoxicity
Testosterone

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Abstract

MTX is a potent cytotoxic drug with applications in cancer treatment via chemotherapy. In addition to suppressing the immune system, methotrexate has hepatotoxic and nephrotoxic side effects, which is one of the leading causes of chronic liver disease and effectively causes testicular damage, one of the major causes of severe health issues in the globe. The present study was designed to investigate the histopathological and biochemical effects of short-term exposure to methotrexate (MTX) on hepatic tissue and testicular tissue; six male rabbits were used. They were divided into two experimental groups: Control-saline and MTX-received groups. MTX was given IP at a daily dose of 50 mg/kg for 14 days. Hepatic and testicular tissue were taken for histological assessment. Blood was collected for serum preparation to demonstrate the testosterone, FSH, and LH hormones. Aspartate aminotransferase AST alanine aminotransferase ALT was evaluated. The microscopic examination of H&E stained liver and testis sections revealed significant congestion and degeneration in the hepatocytes, dilatation of hepatic sinusoids, and hepatitis represented by severe periportal infiltration of mononuclear cells. Vacuolar degeneration in seminiferous tubules, mild morphological changes in spermatogenesis. Also, the results revealed MTX caused significant drops in serum testosterone, FSH, and LH levels in the rabbits, which indicates reproductive injury; serum ALT was significantly increased in the MTX group, and serum AST levels were equivalent between the groups. It is concluded that Methotrexate is a toxic drug, can cause significant histological alterations in hepatic and testicular tissue, and also causes a decrease in testosterone, FSH, LH, and ALT serum levels.

DOI: 10.33899/ijvs.2023.138717.2828,

Introduction

Due to its effectiveness, methotrexate (MTX), a folic acid antagonist, has been utilized in malignant clinical practice for many years as an anticancer medicine (1,2). It has already been reported that methotrexate is toxic to various body organs, including the gastrointestinal, hematologic, and central neurological systems (3,4). The liver is one of the body's most essential organs since it serves as a hub for the metabolism of nutrients like proteins, carbs, and lipids, as well as the excretion of waste products from metabolism (5). Hepatotoxicity can be caused by various risk factors, including hereditary, non-genetic, and environmental ones (6). Anticancer medications, antiretroviral drugs, alcohol, and several medical conditions like age, sex, and diseases all have pharmaceuticals whose metabolites cause liver damage (7). Once administered at some very low doses, methotrexate (MTX) seems to affect the liver and alter its histology.

Malaise, nausea, vomiting, diarrhea, headaches, minor alopecia, and fever are more often reported harmful side effects. A different kind of hepatotoxicity, including cirrhosis, is carried on by low-dose therapy (8). It has been observed that taking MTX causes an increase in aminotransferase levels in the blood and has been associated with the development of liver conditions such as cirrhosis and liver fibrosis (9). Methotrexate's toxic side effects have been seen in various animals, including rats, mice, rabbits, and dogs. Clinically, one of the significant limitations of using methotrexate at the required levels is the hepatotoxicity resulting from long-term use. MTX has been shown to lead to a reduction in the antioxidant enzymes. Due to its antioxidant properties, MTX-induced antioxidant enzyme depletion may contribute to an increase in reactive oxygen species (ROS). Part of the reason for MTX's effects is that it makes more ROS directly, which makes it hazardous. "Oxidative stress" is caused by an imbalance between the generation of ROS and antioxidant defenses, which may result in several clinical and pathological disorders (10), commonly hepatic and renal toxicity (11). The reproductive system has also been implicated in MTX effects. Both human and animal male gonads (testes) undergo cellular alterations (12). Animal studies have demonstrated abnormal spermatogenesis, cytotoxicity, spermatocyte, Sertoli cell, and Leydig cell degeneration (13,14). According to studies, oxidative stress is a key factor in the pathophysiology of MTX-induced testicular injury (15). An increase in reactive oxygen radicals (ROS) has been associated with atrophy in the testicular seminiferous tubules and death in spermatocytes (14,16). The shape of the testis was reportedly disrupted by oxidative stress, which also permanently destroyed the germ cells (17,18). Methotrexate testicular toxicity is a significant adverse effect that might cause infertility.

Materials and methods

Ethical approval

The study was conducted in accordance with the declaration of University of Kerbala, College of Veterinary Medicine, Animal Usage Protocol Committee No.9/30 on 15/6/2022.

Animals and experimental design

Six sexually mature domestic male rabbits aged 8-10 weeks, obtained from the veterinary faculty of Kerbala University animal house, were used for the study. The Animal Ethics Committee of Kerbala University's Veterinary Medicine College approved the set of criteria applied to this work. The body weight of the animals ranged from 1-1.5 kg. The rabbits were given time to acclimate under consistent temperature, humidity, and light/dark cycle conditions. Before the experiment, the animals were given free access to regular food and drinking water. Food was carefully selected and confined to vegetables and lettuce. They were divided into two groups, having three animals in each group. The control group (group 1) was administered normal saline. Methotrexate was administered to rabbits of group 2 at the dose rate of 50 mg per kg body weight intraperitoneally daily for two weeks (19). At the end of the experiment, all the animals were sacrificed, and blood was collected. After scarification, the liver and testis were swiftly extracted and fixed in 10% neutral buffered formaldehyde. Serums were prepared for liver function tests and evaluation of Testosterone and FSH.

Chemicals

Methotrexate 50 mg/ml was obtained from Neova Biogene Pvt Ltd (India).

Serum reproductive hormones and liver markers measurements

The FSH, LH, and testosterone levels were determined using ready-made kits (BT lab, Chinese) and ELISA (Biotech, USA) on serum samples taken from experimental animals. The manufacturer's recommendations examined each hormone. For AST and ALT assessments, using sanymed kits (China), liver activities were evaluated using a spectrophotometric method at a wavelength λ:340 nm and 37°C. The results were represented as U/L.

Histological analysis

Animals in all the groups were sacrificed, and liver and testis samples were collected and fixed in 10% formalin; after fixation, the liver tissues were processed routinely for embedding in paraffin (20). Tissue sections of 4 μm were stained with hematoxylin-Eosin (H&E) to evaluate the parenchymal and stromal structure of the liver tissues, Seminiferous tubules, and testicular interstitial spaces. After being stained with hematoxylin and eosin, the sections were examined under a light microscope for histopathological assessment (21).

Results

Results of liver histopathology

Based on the slides' microscopic descriptions, the normal control group was observed to have a hepatic radial arrangement and stroma (Figure 1), preserved hepatic cells of intact cytoplasm, prominent nucleus, visible portal tracts, and central veins (Figure 2). In MTX group, we noticed significant hepatocyte vacuolar degeneration, considerable sinusoidal dilatation (Figure 3), and intense congestion compared with the control-saline group (Figure 4). A significant infiltration of periportal inflammatory cells and early necrotic features indicates hepatitis. (Figures 5 and 6). In addition to severe bile duct epithelial hyperplasia (Figure 7).

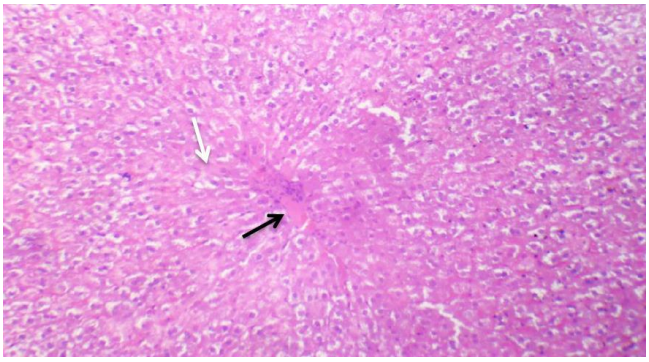

Figure 1: There is vacuolar degeneration of hepatocytes of a control rabbit liver, normal hepatic architecture (white arrow) cords arranged radially around the central vein (black arrow). H&E, 10x.

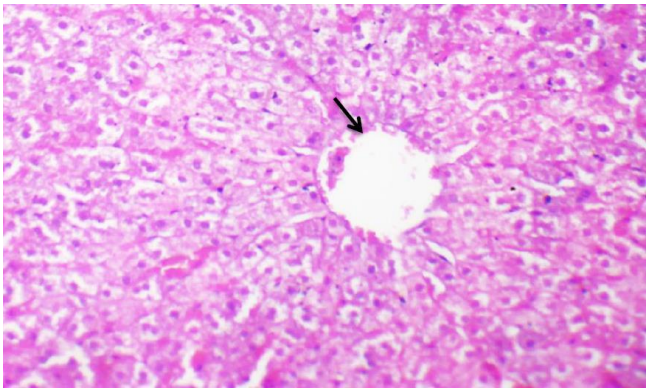

Figure 2: There is vacuolar degeneration of hepatocytes of a control rabbit that showed normal hepatocellular structure, hepatocyte (H) as a large cell with distinct borders and granular cytoplasm around the central vein (C.V) (black arrow). H&E, 40x.

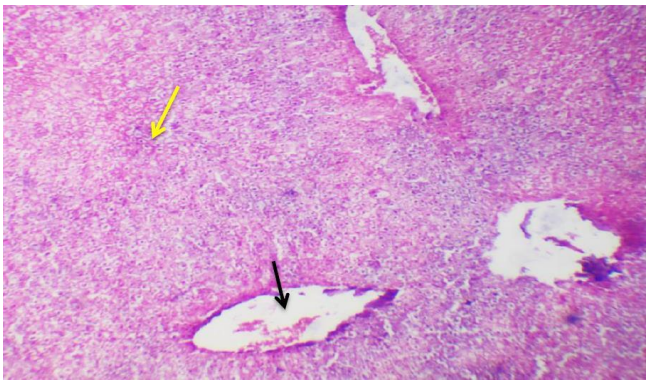

Figure 3: Photomicrograph of the liver of a rabbit treated with 50 mg/kg Methotrexate, showing severe congestion and dilatation of central vein (black arrow), loss of normal architecture (yellow arrow). H&E, 4x.

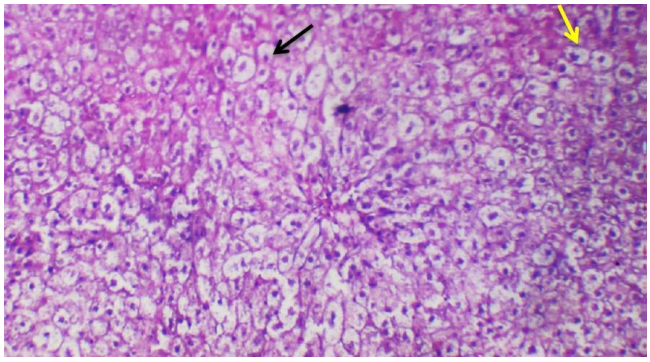

Figure 4: Photomicrograph of a Methotrexate MTX-induced liver histopathological changes revealed significant hepatocellular injury represented by hepatocytes cytoplasmic vacuolation (black arrow). H&E, 10x.

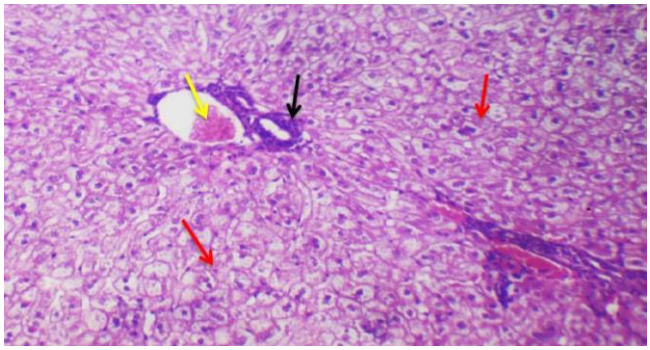

Figure 5: Photomicrograph of a Methotrexate MTX-treated liver tissue revealed marked periportal inflammatory cells infiltration (black arrow), mild to moderate portal vein congestion (yellow arrow), and significant necrotic nuclear changes (red arrow). H&E, 10x.

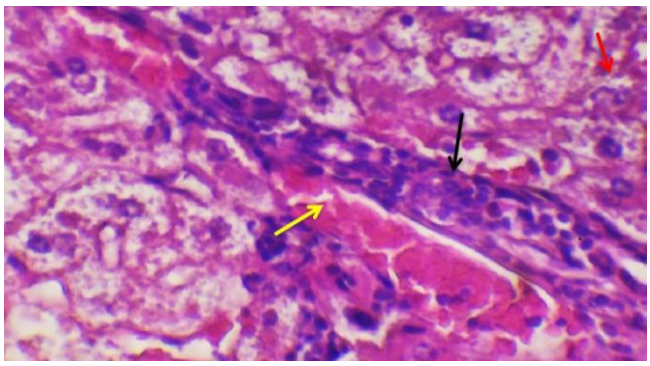

Figure 6: Photomicrograph of a Methotrexate MTX -treated liver tissue, revealed significant, severe chronic periportal inflammatory cells infiltration, mainly macrophages (black arrow), vascular congestion (yellow arrow), and severe hepatocellular necrotic changes manifested by nuclear karryohexis (red arrow). H&E, 40x.

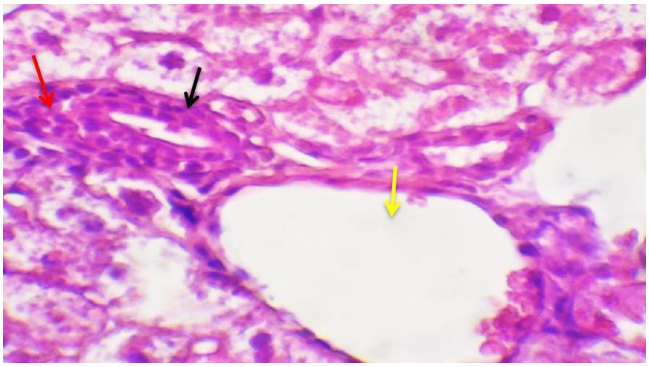

Figure 7: Photomicrograph of a Methotrexate MTX--induced liver tissue injury showed severe bile duct epithelial hyperplasia (black arrow), portal vein dilatation (yellow arrow), and significant inflammation (red arrow). H&E, 40x.

Testicular histopathology

MTX group testes showed histological alterations compared to control group testes, spermatocyte disintegration, and tubular cell necrosis (Figures 8-10). The main histological findings include reduced mature spermatozoa in the lumen edge of the seminiferous tubules and epithelial degeneration, irregular morphology, and deformation of germ cells (Figure 11).

Serum testosterone, FSH, LH, AST and ALT

The figure 12A and 12B demonstrates that MTX-treated rabbits had considerably lower serum FSH LH levels compared to control, which is a symptom of reproductive injury. MTX caused significant drops in serum testosterone levels in rats, a sign of reproductive impairment (Figure 12C). ALT activity in the MTX group was considerably higher than in the Control-saline group (Figure 12E). There was no statistically significant difference in the AST levels across the groups (Figure 12D).

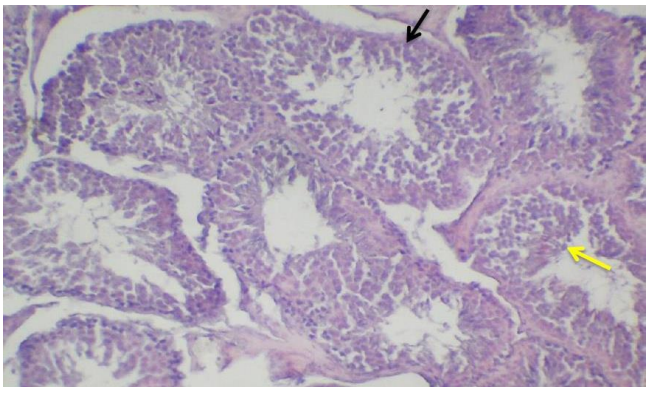

Figure 8: The best Photomicrograph of the testis in the control group showed normal morphology of seminiferous tubules (black arrow); consider the germinal cells' orderly arrangement (yellow arrow). H&E, 10x.

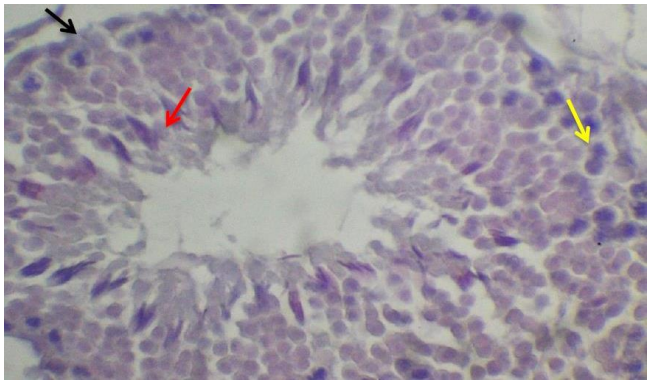

Figure 9: Photomicrograph of testis in the control group, showed normal morphology of seminiferous tubules (black arrow), normal germinal epithelia (yellow arrow), and presence of mature spermatozoa towards tubular lumen (red arrow). H&E, 40x.

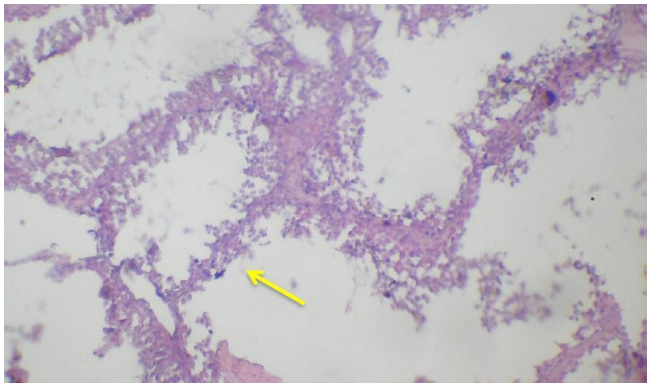

Figure 10: Photomicrograph of testis in MTX group showed disarrangement in seminiferous tubule morphology (black arrow) disruption of germinal epithelia (yellow arrow). H&E, 10x.

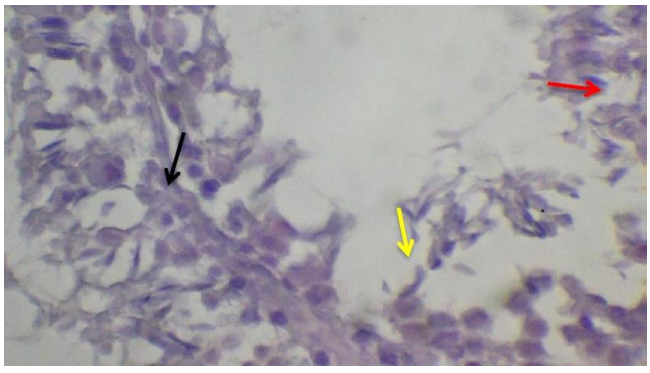

Figure 11: Photomicrograph of testis in MTX group, showing immature germinal epithelia of seminiferous tubules (black arrow), reduction in mature spermatozoa (yellow arrow), and degeneration of spermatocytes (red arrow). H&E, 40x.

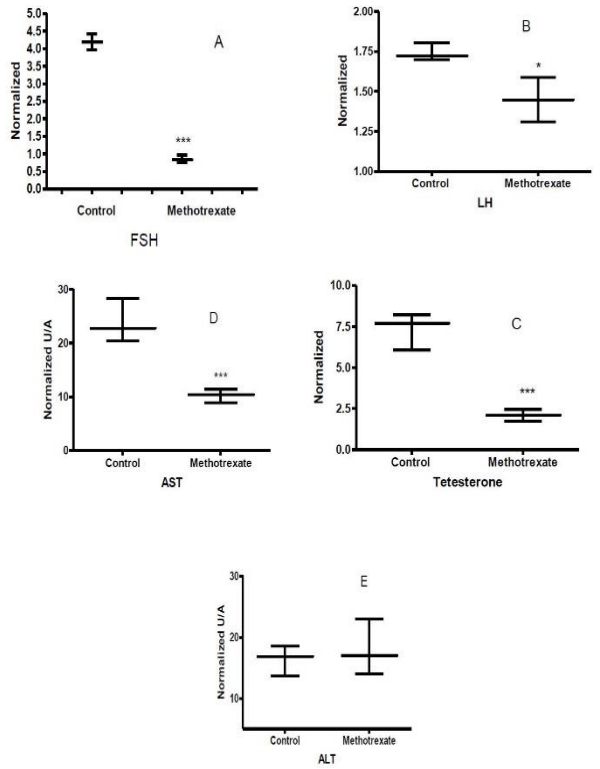

Figure 12: Evaluation of MTX effects on rabbits' serum FSH(A), LH(B), Testosterone(C), AST(D), and ALT(E) levels for the two groups of rabbits: control group (CTRL), MTX-treated group (MTX).

Discussion

One of the drugs used to treat anti-metabolites is MTX, which is recommended for various neoplastic illnesses. However, MTX toxicity has been linked to several organ systems (22). Low dosages of MTX may cause alterations in the liver's histology and, over time, may result in a distinct kind of hepatotoxicity. However, high-dose administration, like in leukemia, can cause severe liver tissue destruction, an increase in hepatic enzymes, cirrhosis, and progressive fibrosis (23,24). Histopathological analysis of the current investigation revealed that MTX significantly caused histopathological modifications and hepatic tissue injury. This was consistent with earlier research (25,26), which discovered that MTX can cause hepatic lesions like focal fibrosis (27). These lesions could be recognized because the liver is the primary site of MTX metabolism into the toxic agent 7-hydroxy-MTX (28,29). Although the precise mechanism by which MTX causes hepatotoxicity is not entirely understood, it can be primarily related to the production of oxidative stress (30). As a result, other papers indicated that taking MTX causes the depletion of antioxidant enzymes and the production of free radicals (31,32), consistent with our findings. According to several studies, administering anti-cancer medications can cause qualitative and quantitative changes in gametes (33,34). Like other chemical products like pesticides, MTX also affected fertility (35-38). It has been documented that giving male rats anticancer medications like cisplatin and Adriamycin results in desquamation of germ cells, degeneration, and a decrease in seminiferous diameter and germinal cell thickness (39,40). The most prevalent long-term morbidities associated with chemotherapy are generally regarded as gonadal dysfunctions connected to chemotherapy and their impact on reproductive health (41). Yet, MTX damages testicular tissue by activating the body's endogenous inflammatory process in the cell (42).

In addition, MTX injection caused testicular injury that was evident in the sloughing, atrophy, and degeneration of germ cells in the seminiferous tubules (43). The current study on rabbits' male reproductive system supports this. Particularly the degeneration of germ cells and reduced spermatogenesis. According to studies (44,45), the etiology of testicular injury caused by MTX is linked to high levels of oxidative stress, which activate the inflammatory response; our results support earlier studies on other tissues that demonstrated MTX to cause severe histological damage and modifications (46,47). Moreover, spermatogenetic abnormalities doubt the progression of spermatogenetic stages due to Sertoli cell destruction (48). In the same line, Nouri *et al.* (49) noted that spermatogenesis in MTX-treated rats had not achieved its full capability and had been altered. These modifications might result from MTX's oxidative activity. Hepatotoxicity is demonstrated by an increase in serum ALT activity in the MTX-treated rabbit group. However, AST in our experiment did not differ significantly across groups. For liver pathology, serum ALT is more specific.

AST is a sign of long-term liver toxicity (50). This means that our study time might not be long enough to detect the AST alteration. MTX can potentially disrupt healthy spermatogenesis and ultimately result in infertility since it speeds up DNA damage, apoptosis, and uncontrolled apoptosis in germ cells. In addition to lowering the number and motility of sperm and the levels of serum testosterone, MTX may also shorten seminiferous tubules and slough the epithelium (51).

Conclusion

Our analysis demonstrates the significance of MTX-induced testicular and hepatic injury. Methotrexate administration caused severe damage-related degenerative changes, such as spermatocyte disintegration and a reduction in mature spermatozoa, hepatic lobular alterations with inflammatory cell infiltration, congestion, and hepatocyte degeneration. To better understand how methotrexate works, additional molecular and ultrastructural research is necessary.

Acknowledgment

I would like to express my special thanks to all the efforts provided throughout the time working on this study and for all the support during the project's completion.

Conflicts of interest

There are no conflicts of interest.

References

1. Famurewa AC, Aja PM, Nwankwo OE, Awoke JN, Maduagwuna EK, Aloke C. *Moringa oleifera* seed oil or virgin coconut oil supplementation abrogates cerebral neurotoxicity induced by antineoplastic agent methotrexate by suppression of oxidative stress and neuro-inflammation in rats. J Food Biochem. 2019;43(3):e12748. DOI: 10.1111/jfbc.12748
2. Sener G, Eksio_glu-Demiralp E, Cetiner M, Ercan F, Yegen BC. Beta-glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects. Eur J Pharmacol. 2006;542(1-3):170-178. DOI: 10.1016/j.e jphar.2006.02.056
3. Ng C, Xiao YD, Lum BL, Han YH. Quantitative structure-activity relationships of methotrexate and methotrexate analogues transported by the rat multispecific resistance-associated protein 2 (rMrp2). Eur J Pharm Sci. 2005;26(5):405-13. DOI: 10.1016/j.ejps.2005.07.008
4. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. Oncologist. 2006;11:694-703. DOI: 10.1634/theoncologist.11-6-694
5. Roy SD, Das S, Shil D, Dutta KN. Herbal hepatoprotective agents: A review. World J Pharm Res. 2012;1(2):87-89. DOI: 10.5455/vetworld.2012.185-192
6. DeLeve L, Kaplowitz N. Prevention and therapy of drug-induced hepatic injury. In: Wolfe M, editor. Therapy of digestive disorders. Philadelphia: WB Saunders; 2000. 334-48 p.
7. Mishra S, Aeri V, Katare DP. Hepatoprotective medication for liver injury. World J Pharm Pharm Sci. 2014;3(5):891-932. [available at]
8. Patel NN, Ghodasara DJ, Pandey S, Ghodasara PD, Khorajiya JH, Joshi BP, Dave CJ. Subacute toxicopathological studies of methotrexate in Wistar rats, Vet World. 2014;7(7):489-495. DOI: 10.14202/vetworld.2014.489-495
9. Durrani K, Zakka FR, Ahmed M, Memon M, Siddique SS, Foster CS. Systemic therapy with conventional and novel immunomodulatory agents for ocular inflammatory disease. Surv Ophthalmol. 2011;56:474-510. DOI: 10.1016/j.survophthal.2011.05.003
10. Coleshowers CL, Oguntibeju OO, Ukpong M, Truter EJ. Effects of methotrexate on antioxidant enzyme status in a rodent model. Med Technol. 2010;24(1):5-9. [available at]
11. Al-Abdaly YZ, Saeed MG, Al-Hashemi HM. Effect of methotrexate and aspirin interaction and its relationship to oxidative stress in rats. Iraqi J Vet Sci. 2021;35(1):151-6. DOI: 10.33899/ijvs.2020.126490.1335
12. Vardi N, Parlakpinar H, Ates B, Cetin A, Otlu A. Antiapoptotic and antioxidant effects of β-carotene against methotrexate- induced testicular injury. Fertil Steril. 2009;92(6):2028-2033. DOI: 10.1016/j.fertnstert.2008.09.015
13. Saxena A, Dhungel S, Bhattacharya S. Effect of chronic low dose of methotrexate on cellular proliferation during spermatogenesis in rats. Arch Androl. 2004;50:33-5. DOI: 10.1080/01485010490250533
14. Nouri HS, Azarmi Y, Movahedin M. Effect of growth hormone on testicular dysfunction induced by methotrexate in rats. Androl. 2009;41(2):105-110. DOI: 10.1111/j.1439-0272.2008.00897.x
15. Armagan A, Uzar E, Uz E, Yilmaz HR, Kutluhan S, Koyuncuoglu HR, Soyupek S, Cam H, Serel TA. Caffeic acid phenethyl ester modulates methotrexate-induced oxidative stress in testes of rat. Hum Exp Toxicol. 2008;27(7):547-52. DOI: 10.1177/0960327108092293
16. Badri SN, Vanithakumari G, Malini T. Studies on methotrexate effects on testicular steroidogenesis in rats. Endocr Res. 2000;26:247-62. DOI: 10.3109/07435800009066165
17. Aitken RJ. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Mol Reprod Dev. 2017;84(10):1039-1052. DOI: 10.1002/mrd.22871
18. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. Nat Rev Urol. 2017;14(8):470-485. DOI: 10.1038/nrurol.2017.69
19. Akacha A, Rebai T, Zourgui L, Amri M. Preventive effect of ethanolic extract of cactus (*Opuntia ficus-indica*) cladodes on methotrexate-induced oxidative damage of the small intestine in Wistar rats. J Cancer Res Ther. 2018;14(10):S779-S784. DOI: 10.4103/0973-1482.174555
20. Al-Akash MA, Rajab HA, Al-Assaf IN. Protective effect of *Urtica dioica* in liver and kidney damages induce by ethylene glycol in rabbits: A histopathological study. Iraqi J Vet Sci. 2022;36(1):167-70. DOI: 10.33899/ijvs.2021.129606.1666
21. Jabber EJ, Sarhat ER, Wadee SA, Sedeeq BI, Sarhat TR. Histological and biochemical evaluation of the effect of desloratadine drug in hepatic tissues. J Hunan Univ Nat Sci. 2021;48(9). [available at]
22. Goujon C, Viguier M, Staumont-Salle D, Bernier C, Guillet G, Lahfa M, Ferrier Le Bouedec MC, Cambazard F, Bottigioli D, Grande S, Dahel K, Berard F, Rabilloud M, Mercier C, Nicolas JF. Methotrexate versus cyclosporine in adults with moderate-to-severe atopic dermatitis: A phase III randomized noninferiority trial. J Allergy Clin Immunol. 2018;6(2):562-569. DOI: 10.1016/j.jaip.2017/.07.007
23. Aboubakr EM, Ibrahim AR, Ali FE, Mourad AA, Ahmad AM, Hofni A. Fasudil ameliorates methotrexate-induced hepatotoxicity by modulation of redox-sensitive signals. Pharm. 2022;15(11):1436. DOI: 10.3390/ph15111436
24. Wang ZH, Zhu D, Xie S, Deng Y, Pan Y, Ren J, Liu HG. Inhibition of rho-kinase attenuates left ventricular remodeling caused by chronic intermittent hypoxia in rats via suppressing myocardial inflammation and apoptosis. J Cardiovasc Pharmacol. 2017;70:102-109. DOI: 10.1097/fjc.0000000000000496
25. Avouac J, Degrave R, Vergneault H, Combier A, Wanono S, Boisson M, Frantz C, Allanore Y. Risk of liver fibrosis induced by methotrexate and other rheumatoid arthritis medications according to the fibrosis-4 index. Clin Exp Rheumatol. 2022;40:150-157. DOI: 10.55563/clinexprheumatol/usddjr
26. Fadel MA, Abdullah MA, Al-Mahmood SS, Thanoon IA. Protective effect of propolis on liver and kidney injury caused by methotrexate in chicks. Iraqi J Vet Sci. 2022;36(4):1067-1. DOI: 10.33899/ijvs.2022.133021.2162
27. Yodoi R, Tamba S, Morimoto K, Segi-Nishida E, Nishihara M, Ichikawa A, Narumiya, S, Sugimoto Y. RhoA/Rho kinase signaling in the cumulus mediates extracellular matrix assembly. Endocrinol. 2009;150:3345-3352. DOI: 10.1210/en.2008-1449
28. Bryan BA, Street CA, Routhier AA, Spencer C, Perkins AL, Masterjohn K, Hackathorn A, Montalvo J, Dennstedt EA. Pharmacological inhibition of Rho-kinase (ROCK) signaling enhances cisplatin resistance in neuroblastoma cells. Int J Oncol. 2010;37:1297-1305. DOI: 10.3892/ijo_00000781
29. Min F, Jia XJ, Gao Q, Niu F, Hu ZY, Han YL, Shi HJ, Yu Y. Remote ischemic post-conditioning protects against myocardial ischemia/reperfusion injury by inhibiting the Rho-kinase signaling pathway. Exp Ther Med. 2019;19:99-106. DOI: 10.3892/etm.2019.8176
30. Abdel-Wahab BA, Ali FM, Alkahtani SA, Alshabi AM, Mahnashi MH, Hassanein EM. Hepatoprotective effect of rebamipide against methotrexate-induced hepatic intoxication: role of Nrf2/GSK-3β, NF-κβ-p65/JAK1/STAT3, and PUMA/Bax/Bcl-2 signaling pathways. Immunopharmacol Immunotoxicol. 2020;42:493-503. DOI: 10.1080/08923973.2020.1811307
31. Maruf AA, O'Brien PJ, Naserzadeh P, Fathian R, Salimi A, Pourahmad J. Methotrexate induced mitochondrial injury and cytochrome c release in rat liver hepatocytes. Drug Chem Toxicol. 2018;41:51-61. DOI: 10.1080/01480545.2017.1289221

32. El-Ghafar OA, Hassanein EM, Ali FM, Omar ZM, Rashwan EK, Mohammedsaleh ZM, Sayed AM. Hepatoprotective effect of acetovanillone against methotrexate hepatotoxicity: Role of Keap-1/Nrf2/ARE, IL6/STAT-3, and NF-_B/AP-1 signaling pathways. Phytother Res. 2022;36:488-505. DOI: 10.1002/ptr.7355
33. Fisch B, Abir R. Female fertility preservation: Past, present and future. Reprod. 2018;156(1):F11-F27. DOI: 10.1530/REP-17-0483
34. Sonigo C, Grynberg M, Bringer S, Sermondade N. Oncofertility and breast cancer. Bull Cancer. 2019;106(12S1):S43-S52. DOI: 10.1016/S0007-4551(20)30047-3
35. Badraoui R, Ben-Nasr H, Bardakci F, Rebai T. Pathophysiological impacts of exposure to an endocrine disruptor (tetradifon) on a-amylase and lipase activities associated metabolic disorders. Pestic Biochem Physiol. 2020;167:104606. DOI: 10.1016/j.pestbp.2020.104606
36. Badraoui R, Sahnoun Z, Abdelmoula NB, Hakim A, Fki M, Rebaï T. May antioxidants status depletion by Tetradifon induce secondary genotoxicity in female Wistar rats via oxidative stress?. Pestic Biochem Physiol. 2007;88(2):149-155. DOI: 10.1016/j.pestbp.2006.10.007
37. Kudavidanage EP, Dissanayake DI, Keerthirathna WR, Nishshanke NW, Peiris LC. Commercial formulation of chlorpyrifos alters neurological behaviors and fertility. Biol. 2020;9(3):49. DOI: 10.3390/biology9030049
38. Peiris LC, Chathu P, Perera DD, Moore HD. 1,3- Dinitrobenze-induced genotoxicity through altering nuclear integrity of diploid and polyploidy germ cells. Dose-Response. 2019;17(3):1559325819876760. DOI: 10.1177/1559325819876760
39. Spears N, Lopes F, Stefansdottir A, Rossi V, De Felici M, Anderson RA, Klinger FG. Ovarian damage from chemotherapy and current approaches to its protection. Hum Reprod Update. 2019;25(6):673-693. DOI: 10.1093/humupd/dmz027
40. Smart E, Lopes F, Rice S, Nagy B, Anderson RA, Mitchell RT, Spears N. Chemotherapy drugs cyclophosphamide, cisplatin and doxorubicin induce germ cell loss in an in vitro model of the prepubertal testis. Sci Rep. 2018;8(1):1773. DOI: 10.1038/s41598-018-19761-9
41. Kawai K, Nishiyama H. Preservation of fertility of adult male cancer patients treated with chemotherapy. Int J Clin Oncol. 2019;24:34-40. DOI: 10.1007/s10147-018-1333-0
42. Wang Y, Zhao TT, Zhao HY, Wang H. Melatonin protects methotrexate-induced testicular injury in rats. Eur Rev Med Pharmacol Sci. 2018;22(21). [available at]
43. Kim JC, Kim KH, Chung MK. Testicular cytotoxicity of DA- 125, a new anthracycline anticancer agent, in rats. Reprod Toxicol. 1999;13(5):391-397. DOI: 10.1016/S0890-6238(99)00028-3
44. Armagan A, Uzar E, Uz E, Yilmaz HR, Kutluhan S, Koyuncuoglu HR, Soyupek S, Cam H, Serel TA. Caffeic acid phenethyl ester modulates methotrexate-induced oxidative stress in testes of rat. Hum Exp Toxicol. 2008;27(7):547-552. DOI: 10.1177/0960327108092293
45. 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;97:1486-92. DOI: 10.1016/j.biopha.2017.11.078
46. Jahovic N, Sener G, Cevik H, Ersoy Y, Arbak S, Yegen BC. Amelioration of methotrexate-induced enteritis by melatonin in rats. Cell Biochem Funct. 2004;22(3):169-178. DOI: 10.1002/cbf.1071
47. Miyazono Y, Gao F, Horie T. Oxidative stress contributes to methotrexate-induced small intestinal toxicity in rats. Scand J Gastroenterol. 2004;39(11):1119-1127. DOI: 10.1080/00365520410003605
48. Taha AN, Ismail HK. The impact of nano zinc oxide particles on the histology of the male reproductive system of adult male rabbits. Iraqi J Vet Sci. 2023;37:105-113. DOI: 10.33899/ijvs.2022.133632.2270
49. Nouri HS, Azarmi Y, Movahedin M. Effect of growth hormone on testicular dysfunction induced by methotrexate in rats. Androl. 2009;41(2):105-110. DOI: 10.1111/j.1439-0272.2008. 00897.x
50. Giannini E, Risso D, Botta F, Chiarbonello B, Fasoli A, Malfatti F, Romagnoli P, Testa E, Ceppa P,Testa R. Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. Arch Intern Med. 2003;163(2):218-24. DOI: 10.1001/archinte.163.2.218
51. Koc F, Erısgın Z, Tekelıoglu Y, Takır S. The effect of beta glucan on MTX induced testicular damage in rats. Biotech Histochem. 2018;93(1):70-5. [available at]

تأثيرات دواء الميثوتريكست على انسجه الكبد والخصى في ذكور الأرانب: دراسة نسيجية مرضية، وهرمونية وكيموحيوية

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الخلاصة

الميثوتريكست علاج فعال سام للخلايا وله تطبيقات علاجية حيث يعتبر العلاج الكيميائي للأورام الخبيثة. بالإضافة الى نشاطه المناعي المثبط وله أثار جانبية تسبب تسمم الكبد، الكلية. تعد السمية الكبدية من الأسباب الرئيسية لأمراض الكبد المزمنة، ويسبب بقوة ضررا في الخصى والتي تعد من المشاكل الصحية الخطيرة في جميع أنحاء العالم. صممت الدراسة الحالية لمعرفة تأثير التعرض قصير المدى لجرعات عالية من الميثوتريكسيت على الأنسجة الكبدية وأنسجة الخصية في ذكور الأرانب. تم استخدام ستة ذكور من الأرانب للدراسة. تم تقسيمهم إلى مجموعتين تجريبية بالتساوي؛ مجموعة السيطرة أعطيت محلول ملحي، مجموعة الميثوتريكسيت أعطيت دواء الميثوتريكسيت بطريقة الحقن داخل الخلب بجرعة واحدة يوميا بمقدار ٥٠ملغم/كجم من وزن الحيوان الواحد ولمدة ١٤ يوما. تم أخذ أنسجة الكبد والخصية للتقييم النسيجي. وتم جمع عينات الدم لتحضير المصل لقياس مستويات هرمون التستوستيرون والهرمون المحفز للقند والهرمون المحفز للجسم الأصفر. فضلا عن قياس مستويات إنزيم الالنين ناقلة أمين الاسبارتيت وإنزيم الأمين ناقلة الألنين. كشف الفحص المجهري لعينات الكبد والخصية المصبغة وجود احتقان وتنكس في الخلايا الكبدية، توسع الجيوب الكبدية وحدوث التهاب الكبد متمثلا بالارتشاح الشديد للخلايا وحيدة النواة حول المنطقة البوابية. كما أبرزت الدراسة وجود تنكس فجوي في الأنابيب المنوية، وتغيرات شكلية خفيفة في تكوين الحيوانات المنوية، وتظهر النتائج أيضا أن دواء الميثوتركسيت يسبب نزول في الهرمون الذكري (التستوستيرون) والهرمون المحفز للجريبات والهرمون اللوتيني التي تدل على الأذى الذي تعرض له الجهاز التكاثري للأرانب. في حين إنزيم ناقلة أمين الألنين يزداد كثيرا في مجموعة الميثوتركسيت مقارنة بمجموعة السيطرة أما إنزيم ناقلة أمين الاسبارتيت يبقى ضمن نفس المستويات. يستنتج من الدراسة أن الميثوتريكسيت دواء سام ويمكن أن يسبب تغييرات نسيجية بارزة في أنسجة الكبد والخصية، بالإضافة الى نزول في مستويات هرمون التستوستيرون والهرمون المحفز للقند والهرمون المحفز للجسم الأصفر.