Original Article

HEALTH SCIENCES
MEDICINE

DOI: 10.32322/jhsm.895444
J Health Sci Med 2021; 4(3): 277-282

Histological changes in methotrexate hepatotoxicity after boron application and evaluation of serum thiol-disulfide balance

Andaç Kipalev Neşelioğlu[1], Gülben Akcan[1], Hilal Nakkaş[1], Salim Neşelioğlu[2], Özcan Erel[2], Oya Evirgen[3], H. Meltem Özgüner[1]

[1]Yıldırım Beyazıt University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Turkey
[2]Yıldırım Beyazıt University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey
[3]Ankara University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Turkey

Cite this article as: Kipalev Neşelioğlu A, Akcan G, Nakkaş H, et al. Histological changes in methotrexate hepatotoxicity after boron application and evaluation of serum thiol-disulfide balance. J Health Sci Med 2021; 4(3): 277-282.

ABSTRACT

Aim: Methotrexate, a folic acid antagonist is a chemotherapeutic drug used in the treatment of various inflammatory diseases as well as some cancer types. The purpose of this study; it is the study of the effects of boric acid against the hepatotoxic side effects of methotrexate.

Material and Method: Male Wistar albino rats were divided into five groups and each group consisted of six animals. The rats in group 1 were used as a control group. Methotrexate was administered to the rats in group 2 and boric acid to the rats in group 3. While the rats in group 4 were given first methotrexate and then boric acid, the rats in group 5 were administered boric acid first and then methotrexate.

Results: Light microscopic examination revealed sinusoidal dilatation, hepatocyte degeneration, vascular congestion-thrombosis, and inflammatory infiltration in the livers of rats treated with methotrexate. It was observed that the protective effect of boric acid was more effective than its treatment. In the groups given methotrexate, the level of oxidative stress-related parameters such as lipid hydroperoxide, MPO and disulfide increased ($p<0.05$ for all parameters), whereas the level of antioxidant parameters such as native thiol, total thiol and catalase decreased ($p<0.05$ for all parameters).

Conclusion: In this study, the protective effect of boric acid was found to be higher than the therapeutic effect in liver damage caused by methotrexate. Oxidative hepatotoxicity resulting from methotrexate application disrupted the thiol disulfide balance and caused a shift to the oxidation side.

Keywords: Boric acid, disulfide, methotrexate, oxidative stres, thiol

INTRODUCTION

Methotrexate (MTX) is a folic acid antagonist and is an antineoplastic and immunosuppressive drug used in the treatment of many types of cancer such as acute lymphoblastic leukemia (ALL), osteogenic sarcoma and lung cancer, and various autoimmune diseases such as rheumatoid arthritis, psoriasis and multiple sclerosis. While it is used in high doses in oncological diseases, a dose of 20mg/m² per week is applied in the treatment of diseases such as ALL and rheumatoid arthritis. MTX affects cells in the synthesis phase. By binding to dihydrofolate reductase, which is the key enzyme in cell replication, it inhibits tetrahydrofolate synthesis, which is necessary for the production of purine and pyrimidine. Thus, by preventing nucleic acid and protein synthesis, it causes the emergence of DNA defects that result in apoptosis (1,2).

In recent studies, the biological importance and positive effects of boric acid (BA) on human health are mentioned. It is emphasized that boric acid plays an important role in immune system, wound healing, energy metabolism, bone development, mineral and hormone metabolism, and antioxidant system (3).

In this study, we aimed to evaluate the protective and/or therapeutic effect of BA against the hepatotoxide effects of MTX, which is widely used in the clinic, and to determine the relationship between these side effects and thiol metabolism.

Corresponding Author: Andaç Kipalev Neşelioğlu, kipalev@gmail.com
Received: 13.03.2021 **Accepted:** 15.04.2021

MATERIAL AND METHOD

This study was conducted with the approval of Ankara University Animal Experiments Local Ethics Committee (Date: 22.01.2020, Decision No: 2020-2-17). All procedures were carried out adhering to the ethical rules and the accordance with the principles of Guide for the Care and Use of Laboratory Animals.

All chemicals and drugs used in the study were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI) and Merck Co. (Darmstadt, Germany). Also, all chemicals were ultrapure grade, and type-I reagent-grade deionized water was used.

Thirty male Wistar Albino rats were used in this study. The rats were fed at room temperature for 12 hours of light (7:00-19:00) and 12 hours of darkness (19:00-7:00). Baits in steel containers; water was given in glass bottles. A single dose of 20 mg/kg methotrexate, which was determined as a result of literature review, was administered (1,2,4,5).

Subjects were divided into 5 groups, each containing 6 rats:

- **Group 1 (Sham group):** Saline injection was administered (for 10 days).
- **Group 2 (MTX group):** Wistar Albino rats were administered 20 mg/kg subcutaneous methotrexate for 1 day to induce hepatotoxicity in the liver. Saline injection was given for the next 9 days
- **Group 3 (BA group):** Wistar Albino rats were injected with 20 mg/kg i.p. BA for 10 days to evaluate the effect of BA on the liver.
- **Group 4 (MTX+BA group):** Wistar Albino rats were administered 20 mg/kg subcutaneous MTX for 1 day and then 20 mg/kg i.p. BA for 9 days to evaluate the healing effect of BA against MTX-induced hepatotoxicity.
- **Group 5 (BA+MTX group):** To evaluate the protective effect of BA against MTX-induced hepatotoxicity, Wistar Albino rats were administered 20 mg/kg i.p. BA for 9 days and then 20 mg/kg subcutaneous methotrexate for 1 day.

On the 11th day, after decapitation under anesthesia (75 mg/kg ketamine+10 mg/kg xylazine), the liver tissues of the rats were removed rapidly by midsaggital incision. It was fixed with 10% formaldehyde. Paraffin blocks were prepared by histological tissue preparation procedure. 4μm thick sections were taken from the paraffin blocks. Sections were stained with Hematoxylin & Eosin (H&E) and Masson Trichrom. The prepared preparations were examined and photographed with an Olympus BX43 photomicroscope. Scoring was done using the H-Score method (6). In addition, various oxidant, antioxidant and, liver function tests were measured in the sera of the rats. All biochemical tests measured in the study were performed on the Siemens ADVIA 1800 Automatic Analyzer (Siemens Healthycare GmbH, Erlangen, Germany). Albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) tests have been studied with commercial kits of this device. Native thiol and total thiol levels were measured and expressed as μmol/L, disulfide and % disulfide/native thiol parameters were calculated according to the method developed by Erel and Neselioğlu (4). Lipid hydroperoxide (LOOH) was measured using the xylenol orange method described by Jiang et al. (5), and the results were expressed as μmol/L. As described by Bradley et al. (6) MPO measurement was performed according to the method used the chromogen as o-dianisidine. Enzyme activity was presented as U/L. Catalase enzyme was measured according to the method described by Goth (7) and the activity results were given as U/L.

A sample size calculation was carried outconsidering detection of 0.70 effect size, α=0.05 and a power of 80.0 % using analysis of variance (one-way ANOVA). The result of the power analysis showed that the minimum number of sample required was 30. The data were evaluated using visual (histograms, probability plots) and statistical methods (Kolmogorov–Smirnov test and Shapiro-Wilktest) to determine whether the data were normally distributed. Descriptive analyses were presented using mean and standard deviation (mean±SD) for the normally distributed variables. As the data were normally distributed, one-way ANOVA were conducted to compare the parameters among groups. An overall 5% type 1 error was used to infer statistical significance. Statistical analyses and figures were performed using the SPSS software version 20 (SPSS Inc. Chicago, IL, USA).

RESULTS

In our study, sinusoids, hepatocytes, sinusoidal cells and portal areas were observed normally in the sections belonging to the control group (**Figure 1** [**A1, A2**]). Vascular congestion, sinusoidal dilatation, mononuclear cell infiltration in the periportal area and hydropic changes in hepatocytes were detected in group 2 in which a single dose of MTX was applied (**Figure 1** [**B1, B2, D1, D2**]). Congestion in the periphery of the lobule and cytoplasmic vacuoles around the nucleus of hepatocytes were observed due to BA applied in group 3. No pathological changes were detected in the vascular structures, and the hepatocyte cell membrane was also preserved (**Figures 1** [**C1, C2**]). There were signs of vascular congestion in group 4, which was applied first MTX and then BA and aimed to observe the therapeutic effect of BA. Mononuclear cell infiltration was found in places in the interstitial area. Hydropic degeneration was observed in hepatocytes around the portal area (at the periphery of the lobule) (**Figure 2 [E1, E2]**). Towards the center, the hepatocyte structure appeared to have been preserved. It was determined that

the degeneration of hepatocytes in group 5, in which BA was applied first and then MTX was applied and the protective effect of BA was aimed to be observed, was much less than group 2 given MTX. It was observed that the hepatic lobule structure was preserved and similar to the control group, and vacuolization was less in hepatocyte cytoplasms. Hepatic fibrosis was not observed in any of the groups (**Figures 2 [F1, F2]**).

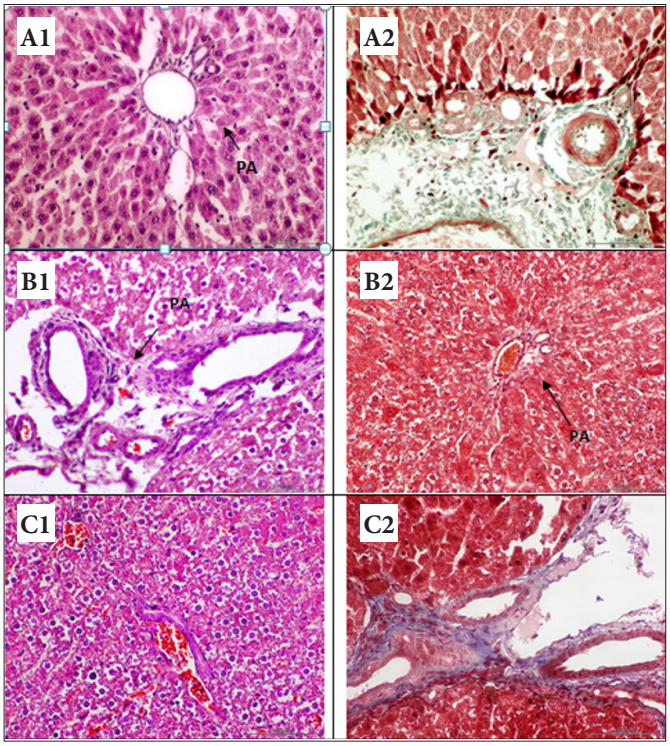

Figure 1. A1 (Group1, control): Hepatocytes, sinusoids and portal area are distinguished normally, H&E x 400, Portal Area (PA). **A2** (Grup1, control): No fibrosis, findings are normal; Masson Trichrom x400. **B1** (Group 2, MTX): Intracellular vacualization, cellular degeneration, capillary vasodilation, cytoplasmic findings are distinguished, H&E x 400. **B2** (Group 2, MTX): There is no increase in collagen fiber, capillary dilatation is present, vascular structures are preserved; Masson Trichrom x 400. **C1** (Group 3, BA): There is no problem in the vascular area, cytoplasmic changes are prominent, there are signs of congestion in the tissue, hydropic degeneration is distinguished, H&E x 400. **C2** (Group 3, BA): Collagen fibers are evident in the vascular area, the vascular structure is preserved, and no endothelial damage has been detected. Masson Trichrom x 400

When groups 2 and 4 are compared with other groups in terms of laboratory parameters; in the groups 2 and 4, the levels of liver function tests (ALT, AST, ALP) and tests reflecting oxidation (disulfide,% disulfide/native thiol, MPO, LOOH) increased ($p<0.05$), antioxidant parameters (native thiol, total thiol, catalase, albumin) levels decreased ($p<0.05$) (**Table, Figure 3**). In addition, evaluating the therapeutic and protective effect of BA; it was determined that all oxidant parameter levels increased and all antioxidant parameter levels decreased in group 4 comparing with healthy controls. In group 5, it was determined that all oxidant parameter levels decreased and all antioxidant parameter levels increased with healthy controls (**Table 1, Figure 3**).

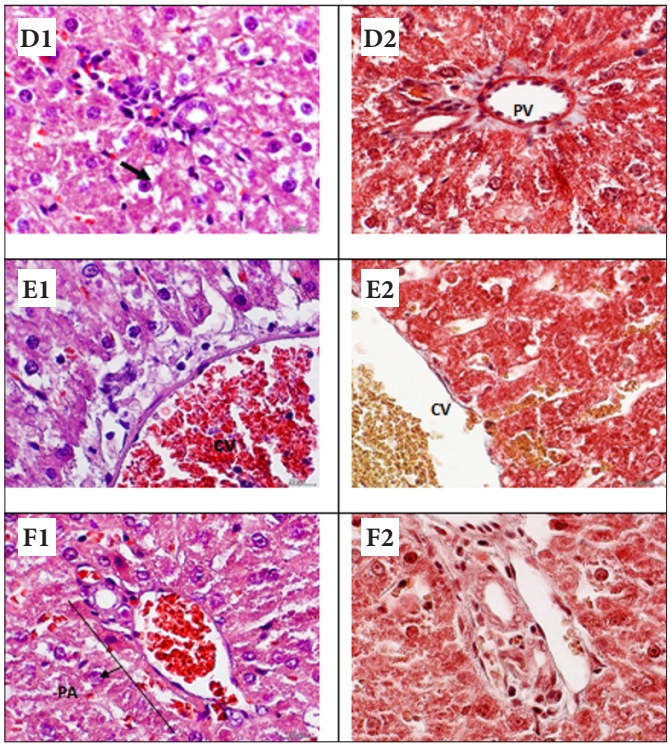

Figure 1. D1 (GROUP 2, MTX): Intracellular vacualization (OC), cellular degeneration, capillary vasodilation, cytoplasmic findings are distinguished, H&E x 1000. **D2** (GROUP 2, MTX): No increase in collagen fiber was detected, capillary dilatation is present, vascular structures are preserved, Masson Trichrome x 1000, Portal Vein (PV) **E1** (GROUP 4, MTX-BA): Signs of vascular congestion are still present, H&E x 1000, Central Vein (CV). **E2** (GROUP 4, MTX-BA): No increase in collagen fiber was detected, Masson Trichrom x 1000 **F1** (GROUP 5, BA-MTX): Less degenerate around the portal area, H&E x 1000, Portal Area (PA). **F2** (GROUP 5, BA-MTX): No fibrosis visible, Masson Trichrom x1000

DISCUSSION

In this study, the process of oxidative stress generating of MTX and the effects of BA against MTX-induced hepatotoxicity were examined (8, 9). MTX is used in the treatment of many cancers and autoimmune diseases due to its antiproliferative, anti-inflammatory, and immunosuppressive properties (8-13). However, many side effects such as hepatotoxicity, small intestine damage, acute renal failure, and lung infiltration have also been reported (14,15). Although the cause of hepatotoxicity due to MTX has been tried to be explained with several possible mechanisms, the main reason is not yet known. One of the possible mechanisms is that MTX causes oxidative stress. In various studies, it has been determined that MTX increases the level of oxidative parameters and reduces the level of antioxidant parameters and it has been stated that hepatotoxicity is due to the increase of ROS (10). In the literature, antioxidants such as methionine, folic acid, and nicotinamide have been used to reduce the side effects of MTX treatment, and these antioxidants have been shown to have protective effects (11-17).

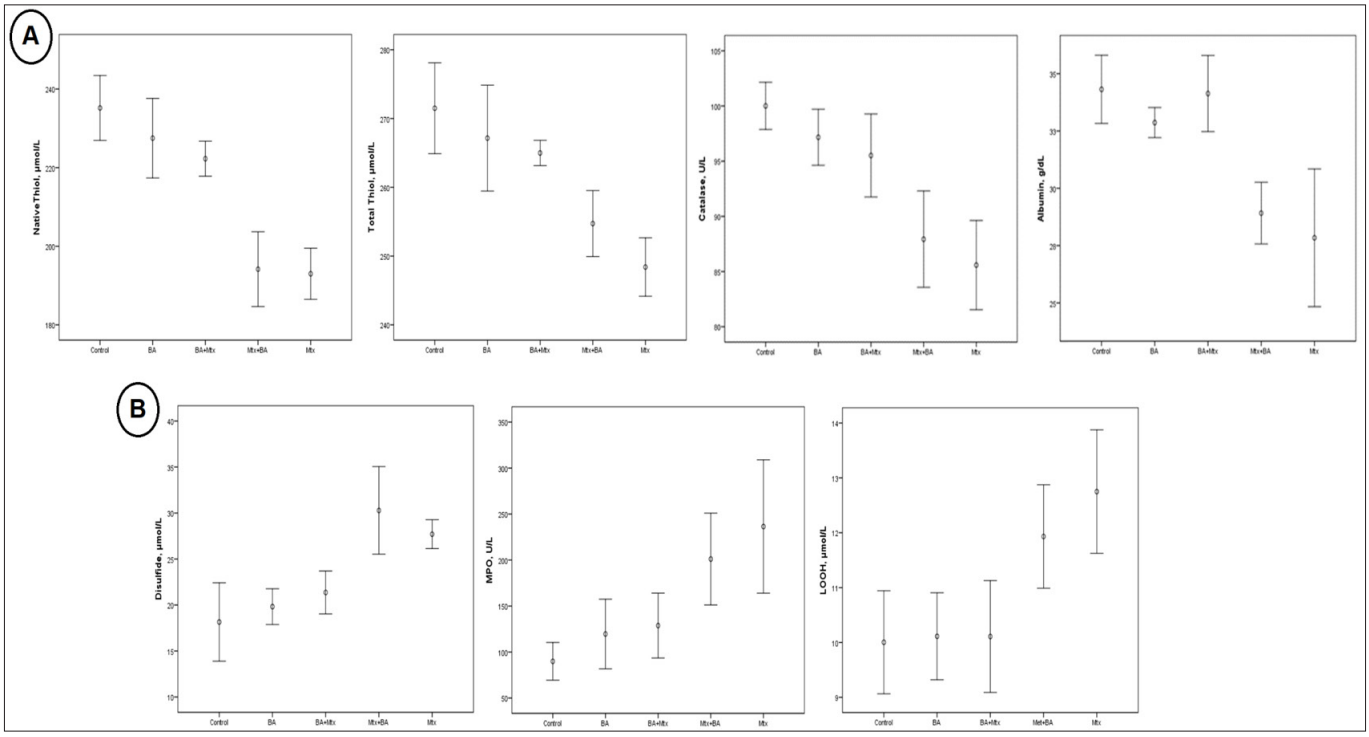

Figure 3. Antioxidant **(A)** and oxidant **(B)** status of the study groups. Data are expressed as mean values±standard error of mean.

Table. Laboratory findings of rats in study groups

Variable	Control (n=6)	BA (n=6)	BA+Mtx (n=6)	Mtx+ BA (n=6)	Mtx	p-value
Native Thiol (µmol/L)	235±7.9[d,e]	228±9.6[d,e]	222±4.2[d,e]	194±9.1[a,b,c]	193±5.2[a,b,c]	<0.001
Total Thiol (µmol/L)	271±6.3[d,e]	267±7.3[d,e]	265±1.8[d,e]	255±4.6[a,b,c]	248±3.4[a,b,c]	<0.001
Disulfide (µmol/L)	18±4.1[d,e]	20±1.8[d,e]	21±2.2[d,e]	30±4.5[a,b,c]	28±1.3[a,b,c]	<0.001
MPO (U/L)	90±19.5[d,e]	120±36.1[d,e]	129±33.6[d,e]	201±47.5[a,b,c]	236±58.4[a,b,c]	<0.001
LOOH (µmol/L)	10±0.9[d,e]	10±0.8[d,e]	10±1.0[d,e]	12±0.9[a,b,c]	13±0.9[a,b,c]	<0.001
Catalase (U/L)	100±2.0[d,e]	97±2.4[d,e]	96±3.6[d,e]	88±4.2[a,b,c]	86±3.3[a,b,c]	<0.001
Albumin (g/dL)	34±1.4[d,e]	33±0.6[d,e]	34±1.6[d,e]	29±1.3[a,b,c]	28±2.4[a,b,c]	<0.001
ALT (U/L)	45±9.7[d,e]	47±6.0[d,e]	49±13.6[d,e]	105±19.8[a,b,c]	108±9.8[a,b,c]	<0.001
AST (U/L)	59±8.9[d,e]	62±12.2[d,e]	66±11.4[d,e]	171±47.8[a,b,c]	189±39.9[a,b,c]	<0.001
ALP (U/L)	78±4.0[d,e]	73±20.3[d,e]	81±18.6[d,e]	180±36.3[a,b,c]	174±38.3[a,b,c]	<0.001

Values are expressed as mean±SD. p value, One-way analysis of variance [ANOVA];
[a]Statistically significant difference between the control group vs other group;
[b]Statistically significant difference between BA (Boric acid) group vs other group;
[c]Statistically significant difference between BA+Mtx (Boric acid+Methotrexate) group vs other group;
[d]Statistically significant difference between Mtx+BA (Methotrexate+Boric acid) group vs other group;
[e]Statistically significant difference between Mtx (Methotrexate) group vs other group;
MPO, myeloperoxidase; LOOH, lipid hydroperoxide; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

MTX is stored in hepatocytes by forming polyglutamate derivatives from folic acid. Polyglutamate derivatives are toxic and cause hepatocyte necrosis. In addition, MTX inhibits dehydrogenases that provide the synthesis of NADPH in cells (17). Thus, intracellular antioxidant NADPH levels decrease and the resulting oxidative stress causes hepatotoxicity (18, 19). In addition, the glutathione reductase enzyme also NADPH is used, which protects the glutathione level against reactive oxygen species (ROS). That is, the decrease in NADPH levels causes a decrease in the synthesis of glutathione, a powerful antioxidant. Thus, hepatocytes become sensitive to oxidation and hepatocyte necrosis develops. As a result, it has been demonstrated that the liver damage caused by methotrexate is mainly caused by oxidative stress (20, 21).

Boric acid, a natural mineral, has been shown to have antioxidant (22) and hepatoprotective (23) effects in many studies. It has also been suggested that BA increases the amount of glutathione in the body, inhibiting other reactive oxygen species and counteracting oxidative damage (24).

Two important results of this study draw attention. First, when group 2 and group 4 were evaluated histologically, it was observed that hepatotoxic damage such as sinusoidal dilatation, hepatocyte degeneration, and intracellular vacuolization occurred in both groups (**Figure 2 [D1, D2, E1, E2]**). When the laboratory parameters of group 2 and group 4 were evaluated, it was found that the levels of tests reflecting hepatocyte damage (ALT,

AST, and ALP) increased ($p<0.05$), the levels of oxidant parameters (lipid hydroperoxide, MPO, disulfide and, % disulfide/native thiol ratio) increased ($p<0.05$) and the levels of antioxidant parameters (native thiol, total thiol, albumin and, catalase) decreased ($p<0.05$) in both groups comparing with other groups. In other words, these detected results show that MTX causes hepatocyte damage and oxidative stress (**Table, Figure 3**). We think that the main reason for the impairment of oxidant-antioxidant balance may be due to the deterioration of thiol-disulfide homeostasis. Thiol disulfide homeostasis consists of antioxidant and oxidant parts and is in equilibrium under physiological conditions. While the native thiol level reflects the antioxidant side of this balance, the disulfide level reflects the oxidant side. Thiol groups can be oxidized for various reasons and thus disulfide bonds are formed. Disulfide bonds can also be converted back to thiol groups by reduction. In another word, thiol-disulfide homeostasis is reversible and in balance. This balance is disturbed in many illness situations (4,25-28). In our study, the decrease in native thiol level and increase in disulfide and % disulfide/native thiol levels in MTX administered groups show that thiol-disulfide balance deteriorates in favor of oxidation (**Table, Figure 3**).

In addition, previous studies required the evaluation of many oxidant and antioxidant parameters in order to quantitatively measure the oxidative stress caused by methotrexate (9,29,30) However, in our study, both antioxidant and oxidant levels can be determined simultaneously by measuring only thiol-disulfide balance. Our work is unique in this respect.

When the laboratory parameters are evaluated, the second important result of our study is that boric acid has a protective effect but does not have a therapeutic effect against hepatocyte damage due to oxidative stress caused by MTX. It has been observed that boric acid given before MTX injection (group 5) has a protective effect by preventing oxidative damage caused by MTX. In addition, it was clearly observed that boric acid administered after MTX administration did not eliminate the oxidative damage caused by MTX and had no therapeutic effect (Table 1, Figure 3). Determining that boric acid is protective and forecasting that cell damage after oxidative stress is mainly caused by the disruption of the thiol-disulfide homeostasis has been a guide to prevent the side effects of methotrexate application. We think that thiol-containing drugs or reinforcing agents with antioxidant properties can be used to prevent the side effects of MTX. Glutathione and N-acetyl cysteine are frequently preferred in many diseases due to their antioxidant and hydrophilic properties (31-33).

CONCLUSION

When histological imaging and laboratory parameters are examined, MTX administration causes damage due to oxidative stress in both blood and liver cells. Therefore, we anticipate that the use of α-lipoic acid, a thiol-containing agent that has both hydrophilic (against oxidation in the blood) and lipophilic (against oxidation in the hepatocytes) property, may be more effective.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was conducted with the approval of Ankara University Animal Experiments Local Ethics Committee (Date: 22.01.2020, Decision No: 2020-2-17).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study had received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and approved the final version.

REFERENCES

1. Güven E, Erişgin Z, Tekelioğlu Y. Siçanlarda metotreksat kaynakli testis hasari üzerine shilajitin'in histopatolojik etkisi. Kocatepe Tıp Derg 2017; 18: 1-6.
2. Uygur Ag, Şingirik E, Yücel AH. Metotreksat kaynaklı hasarlarda alfa lipoik asit'in koruyucu etkisi. Arşiv Kaynak Tarama Derg 2019; 28: 227-36.
3. Ince S, Keles H, Erdogan M, Hazman O, Kucukkurt I. Protective effect of boric acid against carbon tetrachloride–induced hepatotoxicity in mice. Drug and chemical toxicology 2012; 35: 285-92.
4. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014; 47: 326-32.
5. Jiang ZY, Woollard AC, Wolff SP. Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids 1991; 26: 853-6.
6. Thike AA, Chng MJ, Tan PH, Fook-Chong S. Immunohistochemical expression of hormone receptors in invasive breast carcinoma: correlation of results of H-score with pathological parameters. Pathology 2001; 33: 21-5.
7. Góth L. A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta; Int J Clin Chem 1991; 196: 143-51.
8. Tilling L, Townsend S, David J. Methotrexate and hepatic toxicity in rheumatoid arthritis and psoriatic arthritis. Clin Drug Invest 2006; 26: 55-62.
9. Devrim E, Çetin R, Kılıçoğlu B, Imge Ergüder B, Avcı A, Durak İ. Methotrexate causes oxidative stress in rat kidney tissues. Renal Failure 2005; 27: 771-3.
10. Elsawy H, Algefare AI, Alfwuaires M, et al. Naringin alleviates methotrexate-induced liver injury in male albino rats and enhances its antitumor efficacy in HepG2 cells. Bioscience Reports 2020; 40(6).

11. Ulukaya S. Hepatobiliyer Hastalıklarda Anestezi. JCAM 389-99.
12. Armagan I, Bayram D, Candan IA, et al. Effects of pentoxifylline and alpha lipoic acid on methotrexate-induced damage in liver and kidney of rats. Environmental toxicology and pharmacology 2015; 39: 1122-31.
13. Surai P. Carnitine Enigma: from antioxidant action to vitagene regulation part 2. transcription factors and practical applications. J Veter Sci Med 2015; 3: 17.
14. Wang B, Chen M-S, Cao T, et al. editors. Method development for determining methotrexate in urine by micellar electrokinetic capillary chromatography. Medicine and Biopharmaceutical: Proceedings of the 2015 International Conference; 2016: World Scientific.
15. Bordbar M, Shakibazad N, Fattahi M, Haghpanah S, Honar N. Effect of ursodeoxycholic acid and vitamin E in the prevention of liver injury from methotrexate in pediatric leukemia. The Turkish Journal of Gastroenterology 2018; 29: 203.
16. Kılıc S, Emre S, Metin A, Isıkoglu S, Erel O. Effect of the systemic use of methotrexate on the oxidative stress and paraoxonase enzyme in psoriasis patients. Archives of dermatological research 2013; 305: 495-500.
17. Babiak RM, Campello AP, Carnieri EG, Oliveira MB. Methotrexate: pentose cycle and oxidative stress. Cell Biochem Funct 1998; 16: 283-93.
18. Uraz S, Tahan V, Aygun C, et al. Role of ursodeoxycholic acid in prevention of methotrexate-induced liver toxicity. Dig Dis Sci 2008; 53: 1071-7.
19. Jahovic N, Çevik H, Şehirli AÖ, Yeğen BÇ, Şener G. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. J Pineal Res 2003; 34: 282-7.
20. Armağan İ. Metotreksat'ın karaciğer ve böbrekte neden olduğu hasarda oksidatif stresin rolü. SDÜ Tıp Fakültesi Derg 2015; 22.
21. Mercan U. Toksikolojide serbest radikallerin önemi. Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Derg 2004; 15: 91-6.
22. Sogut I, Paltun SO, Tuncdemir M, Ersoz M, Hurdag C. The antioxidant and antiapoptotic effect of boric acid on hepatoxicity in chronic alcohol-fed rats. Canadian J Physiol Pharmacol 2018; 96: 404-11.
23. Güneş E, Büyükgüzel E. Oxidative effects of boric acid on different developmental stages of Drosophila melanogaster Meigen, 1830 (Diptera: Drosophilidae). Türkiye Entomoloji Derg 2017; 41: 3-15.
24. Cengiz M. Boric acid protects against cyclophosphamide-induced oxidative stress and renal damage in rats. Cell Molecul Biol 2018; 64: 11-4.
25. Micoogullari U, Karatas OF, Kisa E, et al. Thiol/disulfide homeostasis in patients with erectile dysfunction. J Sex Med 2020; 17: 1934-41.
26. Sezgin B, Kinci MF, Pirinççi F, et al. Thiol-disulfide status of patients with cervical cancer. J Obstet Gynaecol Res 2020; 46: 2423-9.
27. Erel Ö, Neşelioğlu S, Ergin Tunçay M, et al. A sensitive indicator for the severity of COVID-19: thiol. Turk J Med Sci 2020.
28. Efe A, Neşelioğlu S, Soykan A. An Investigation of the dynamic thiol/disulfide homeostasis, as a novel oxidative stress plasma biomarker, in children with autism spectrum disorders. Autism Res 2021; 14: 473-87.
29. Elango T, Dayalan H, Gnanaraj P, Malligarjunan H, Subramanian S. Impact of methotrexate on oxidative stress and apoptosis markers in psoriatic patients. Clin Exp Med 2014; 14: 431-7.
30. Moghadam AR, Tutunchi S, Namvaran-Abbas-Abad A, et al. Pre-administration of turmeric prevents methotrexate-induced liver toxicity and oxidative stress. BMC Complement Altern Med 2015; 15: 246.
31. Akşit H, Akşit D, Bildik A, Hatibe K, Yavuz Ö, Seyrek K. Deneysel karaciğer intoksikasyonunda N-asetil sistein'in glutatyon metabolizması ve lipid peroksidasyonuna etkileri. Ankara Üniversitesi Veteriner Fakültesi Derg 2015; 62: 1-5.
32. Gül M, Ayan M, Seydanoğlu A, et al. The effect of N-acetyl cysteine on serum glutathione, TNF-α and tissue malondialdehyde levels in the treatment of sepsis. Turk J Trauma Emerg Surg 2011; 17: 293-7.
33. Ommati MM, Amjadinia A, Mousavi K, Azarpira N, Jamshidzadeh A, Heidari R. N-acetyl cysteine treatment mitigates biomarkers of oxidative stress in different tissues of bile duct ligated rats. Stress 2020: 1-16.