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The effect of barbaloin against oxidative liver damage induced by methotrexate in the rats

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Abstract

Methotrexate (MTX) inhibits mitosis by antagonizing the folic acid required for DNA synthesis. Since it is metabolized in the liver, it may cause toxic effects. In this study, the effect of barbaloin (BAR), an antioxidant and anti-inflammatory substance, against MTX-induced liver damage was investigated. Totally 18 animals were divided into control, MTX, and MTX+BAR groups. The study was terminated after liver and blood samples were taken. While liver malondialdehyde (MDA) and myeloperoxidase (MPO) levels raised in the MTX group compared to the control group, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities decreased. While MDA and MPO levels decreased in the MTX+BAR group compared to the MTX group, SOD, CAT, and GPx activities increased. The levels of blood aspartate aminotransferase and alanine aminotransferase increased in the MTX group but decreased in the MTX+BAR group. MTX has toxic effects on the liver and this toxicity diminished with the application of BAR.

Keywords: Barbaloin, liver, methotrexate, oxidative stress, rat

Introduction

The liver is the basic organ that detoxified drugs, alcohol, and metabolic products, which may have numerous toxic effects on the human body. But, prolonged exposure to chemotherapeutic drugs, alcohol, and infections can start liver damage, and even lead to serious liver diseases and liver failure [1]. For this reason, the liver is more sensitive to damage than the other organs with the role in the metabolism of the organism from the perspective ability to concentrate and biotransform xenobiotics and various drugs [2].

Methotrexate (MTX) as a dihydrofolate reductase inhibitor that induces apoptosis in especially cancer cells is a drug that is generally used in the clinic for different cancers and ailments including psoriasis, rheumatoid arthritis. Using of MTX long-term as clinically is restricted because of the reason different organ damage such as hepatotoxicity, and nephrotoxicity depend on chronically high dose administration of MTX. The researchers concentrated on methotrexate due to liver toxicity caused by methotrexate used in many medical cases [3,4]. Furthermore, it has been proposed various mechanisms including oxidative stress explain methotrexate-induced liver damage [4,5].

It was reported that ROS are continuously generated under normal physiological conditions [6,7]. They can rapidly start the membrane polyunsaturated lipids peroxidation. Thus, they lead to the production of lipid peroxides [8-10]. Also, they can harm vital biomolecules like lipids, nucleic acids, polyunsaturated fatty acids, proteins, and carbohydrates. Also, they lead to DNA damage that may cause mutations [11-14]. It is a view accepted by scientific researchers that MTX induces toxicity related to the production of free radicals and oxidative stress response. Thus, it was realized that MTX reasons the oxidative DNA damage and initiates lipid peroxidation. Intensive production of oxygen free radicals raised the MTX hepatotoxicity. Therefore, it exhausts cellular non-enzymatic and enzymatic antioxidant preserve systems [15,16]. The last study findings showed that MTX markers related to diminished or insufficient cellular antioxidant guard system

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finalizing in liver free radical injury [17,18]. Choosing the different non-toxic cytoprotective factors as helping can play an important effect to lessen the density of side effects of MTX chemotherapy with the hindrance of chemotherapeutic effects. Body preservation for toxicity led by substances with chemotherapeutic impacts is accepted as a very significant target and the aim of most research about supporting antioxidant therapy [4]. Using the native products or pills non-toxic cytoprotective substances as enhancing therapeutic effects can play an important role in reducing the rate of toxicity of MTX with the protection of drug effectiveness.

Barbaloin is a specific extract of aloe and is determined that it has several pharmacological effects like anti-inflammatory, free radical scavenging, antiviral, and antibacterial properties. Barbaloin attenuated inflammation, and radical stress and thus prevented hepatic hazardous induced by alcohol for mice experimental models. [19].

As a result of our literature review, we can say that there is no study suggesting the use of BAR in the MTX-induced liver toxicity model. In this respect, we designed this research to measure the possible impacts of BAR on MTX- stimulated liver injury.

Materials and Methods

Ethical Statement

This study was approved by the Ataturk University Animal Experiments Local Ethics Committee (Approval no: 75296309-050.01.04-E.2100036416).

Animals Groups

Eighteen males weighing 190-210g of Wistar Albino rats were supplied from Atatürk University, Laboratory Experimental Animals Research Centre, and in laboratory conditions such as humidity (55%) controlled room, temperature (25°C), normal daylight period cycle. Rats were fed classic traditional feeding provender and water ad libitum. Animals were weighed. After that 3 groups of rats (n=6) randomly were chosen.

Experimental Groups and Protocols

1. Physiological serum was applied to the control group of rats. No other medication was applied.
2. Methotrexate was used single dose as intraperitoneal 20mg. kg^{-1} to methotrexate group.
3. It was used as 20mg/kg/day; as intraperitoneal for 5 consecutive days in the MTX+ BAR group; BAR was received to this group following methotrexate application (20mg/kg/day, single-dose).

Animals were then anesthetized end of the 5th day of the experiment. After a sacrifice by a high dose of thiopental sodium (300mg/kg, i.p.), the liver tissue and serum specimens were taken. The samples were immediately analyzed. The liver tissue samples were washed in cold saline and waited at -80°C until the experiment for MPO, MDA levels, SOD, CAT, and GPx activities. Samples from rats were centrifuged at 5000 rpm for 10 minutes. Haemolysed-free blood sera were transferred to Eppendorf's and stored at -80 °C until the day of analysis. The serum sample levels of AST and ALT were evaluated by traditional ELISA Kits by handling the laboratory equipment from (Olympus Instruments, Tokyo, Japan).

MTX and BAR administration doses were preferred based on the doses used in former studies in which hepatotoxic dose was determined [4,19].

Chemicals

Thiopental sodium, which was used in the applications, was provided from IE Ulagay-Turkey, Barbaloin from Sigma Chemical Co., USA. Methotrexate (methotrexate, 500mg/20mL, Koçak Farma, İstanbul, Turkey).

Biochemical analyses

MDA, MPO, SOD, GPx, CAT, ALT, AST Analysis, and Protein Determination

Frozen liver tissue, which was one hundred milligrams was homogenized in phosphate buffer by a homogenizer. The centrifugation of homogenized tissues was made at 3000 rpm for 15 min. and after the upper part of the samples was removed. For oxidative stress agents, the supernatant taken from the hepatic samples was used. Lipid peroxidation levels were evaluated to estimate the MDA levels of the samples [20]. Shortly, The MDA level, one of the indices of lipid peroxidation, was measured by a method based on the reaction with thiobarbituric acid (TBA) at 90–100°C. In addition, the protein amount of the homogenates was studied according to the method determined by Lowry et al. [21]. Briefly, the protein levels were measured in the homogenate, supernatant, and extracted tissue samples. The Lowry protein assay is a biochemical assay for determining the total level of protein in a solution. The total protein concentration is exhibited by a color change of the sample solution in proportion to protein concentration, which can then be measured using colorimetric techniques. SOD activity was evaluated by using the method of Sun et al. [22]. In brief, the total (Cu–Zn and Mn) SOD activity was measured from extracted tissue samples based on the inhibition of nitroblue-tetrazolium reduction by the xanthine–xanthine oxidase system as a superoxide generator. CAT activity was calculated by using Aebi's method [23]. The activity of CAT was analyzed according to the H_2O_2 decomposition rate. GPx activity was analyzed according to Paglia and Valentine method [24]. Shortly, according to this method, the decrease in the NADPH absorbance at 340 nm, in the presence of H_2O_2, is proportional to GPx enzymatic activity. MPO levels were evaluated with a 4-amino antipyrine/phenol solution. Absorbance changes at 510 nm were recorded [25]. MPO activity was determined using 4-amino antipyrine/phenol solution as the substrate for MPO mediated oxidation by H_2O_2. The analysis of ALT and AST were evaluated by the use of a traditional kit.

Statistical Analysis

Findings were given as Means±S.D. ANOVA and Tukey's test has been used for statistical analyses. Changes for P-value <0.05 were noted as important. The distribution of parameters in the groups was evaluated with the Shapiro Wilks test. Accordingly, the assumption of normality was provided in the groups. Since the assumption of normality was provided, the One-Way Analysis of

Variance test, which is a parametric test, was performed. Tukey test, which is one of the most powerful pairwise tests, was used for comparisons.

Results

While the MDA level was statistically significantly higher in the MTX group compared to the control group, it decreased significantly in the BAR group compared to the MTX group (Figure 1), (P<0.05). While the MPO level in the MTX group increased statistically significantly compared to the control group, it decreased significantly in the BAR group compared to the MTX group (Figure 2), (P<0.05). While the GPx level decreased statistically significantly in the MTX group compared to the control group, it increased significantly in the BAR group compared to the MTX group (Figure 3), (P<0.05). SOD level decreased statistically significantly in the MTX group compared to the control group, while it increased significantly in the BAR group compared to the MTX group (Figure 4), (P <0.05). While CAT level decreased statistically significantly in the MTX group compared to the control group, it increased significantly in the BAR group compared to the MTX group (Figure 5), (P <0.05).

While AST and ALT levels were statistically higher in the MTX group compared to the control group, they were significantly decreased in the BAR group compared to the MTX group (Figure 6), (P<0.05).

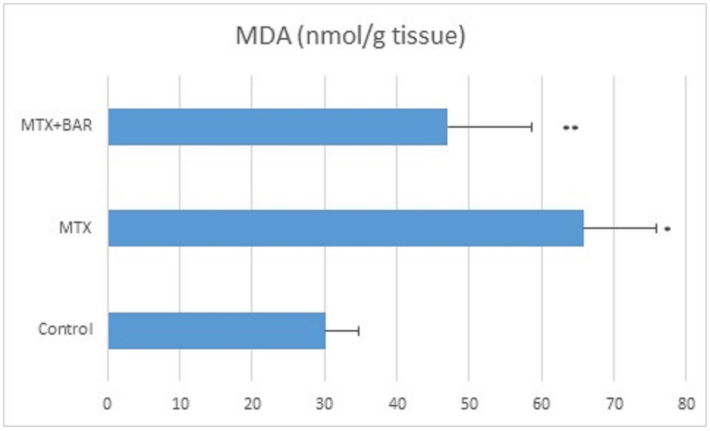

Figure 1. Rat's hepatic tissue MDA (nmol/g tissue) levels. Effect of BAR on MTX-induced liver injury. *P<0.05, comparison to control and BAR+MTX; **P<0.05, comparison to control and MTX treatment. Values presented the mean ± S.D. (n=6)

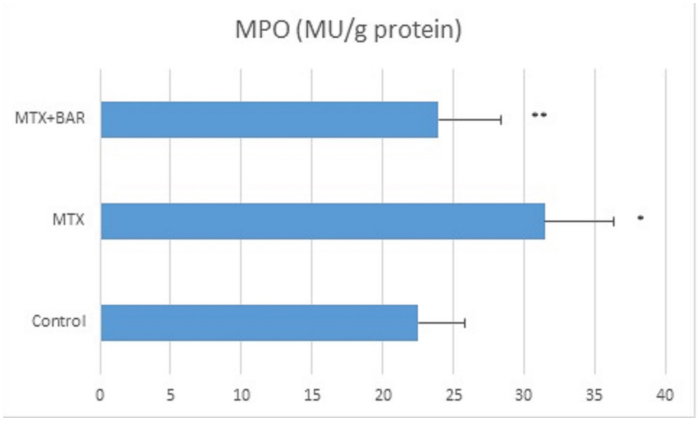

Figure 2. Rat's hepatic tissue MPO (mU/g protein) levels. Effect of BAR on MTX-induced hepatotoxicity. *P < 0.05, comparison to control and BAR+MTX; **P<0.05, comparison to control and MTX treatment. Values presented the mean±S.D. (n=6)

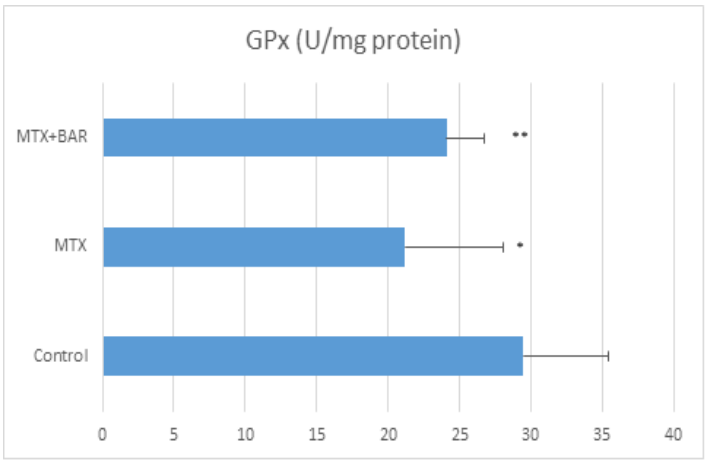

Figure 3. Rat's hepatic tissue GPx (U/mg protein) activities. Effect of BAR on MTX-induced hepatotoxicity. *P<0.05, comparison to control and BAR+MTX; **P<0.05, comparison to control and MTX treatment. Values presented the mean ±S.D. (n=6)

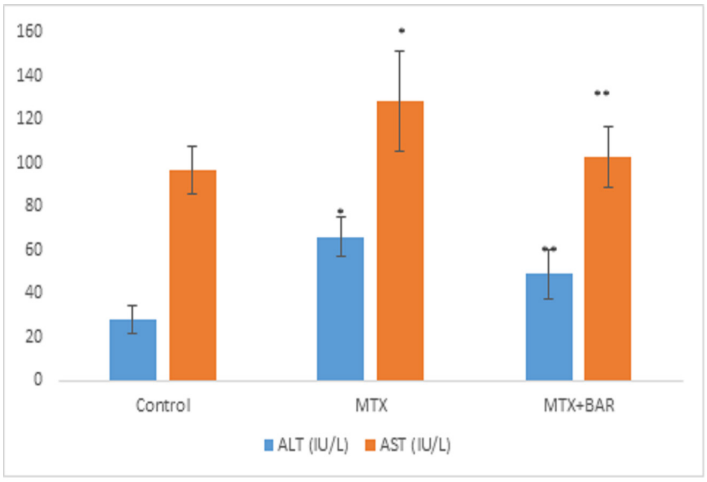

Figure 4. Rat's hepatic tissue SOD (U/mg protein) activities. Effect of BAR on MTX-induced hepatotoxicity. *P < 0.05, comparison to control and BAR+MTX; **P<0.05, comparison to control and MTX treatment. Values presented the mean±S.D. (n=6)

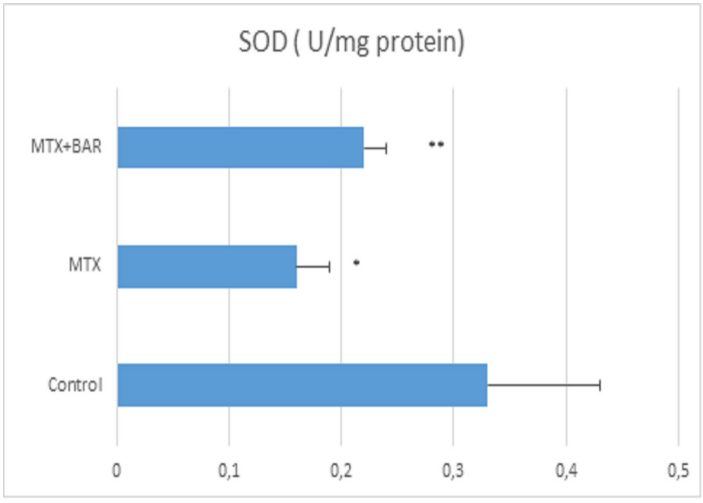

Figure 5. Rat's hepatic tissue CAT (k/g protein) activities. Effect of BAR on MTX-induced hepatotoxicity. *P<0.05, comparison to control and BAR+MTX; **P<0.05, comparison to control and MTX treatment. Values presented the mean ±S.D. (n=6).

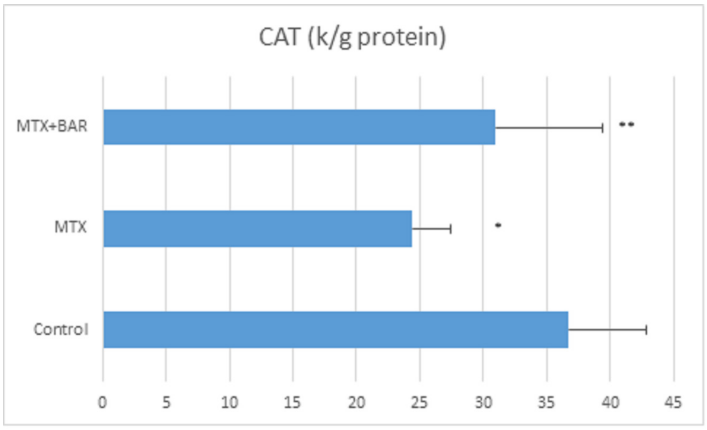

Figure 6. Rat's blood AST/ALT (U/L) levels. Effect of BAR on MTX-induced hepatotoxicity. *P<0.05, comparison to control and MTX+BAR; **P<0.05, comparison to control and MTX treatment. Values presented the mean ± S.D. (n=6).

Discussion

MTX as a highly effective antineoplastic substance can be used the cure many clinical conditions involving various inflammatory ailments. It shows an immunosuppressive impact on some illnesses like lymphoma, rheumatoid arthritis, choriocarcinoma, and leukemia. Also, MTX is used alone or in alliance with other cytotoxic substances in surgery for neoplastic disease, radiotherapy, hormonotherapy, and chemotherapy [26]. The generation of injurious ROS is induced by different pathological processes [27]. Deterioration in favor of ROS of this equilibrium state is defined as oxidative stress [28]. Even though the mechanism of MTX-stimulated liver injury is not comprehended, the most accepted mechanism of the MTX-stimulated various organ injury is oxidant stress caused by ROS [29-31]. MTX toxicity is connected with liver mitochondria injury via abatement of mitochondrial non-enzymatic and enzymatic antioxidants [16]. Researches propose that MTX can restrict the intracellular quantity of NADPH, which is used by GSH reductase to preserve the decreased ratio of cellular GSH. Also, it has been shown that the depletion or reduction of glutathione, induced by MTX, destroys the antioxidant defense system against ROS attacks [29]. By using this pathway, MTX reasons intense membrane damage. An important cellular defense system against ROS is provided owing to antioxidant enzymes like SOD, CAT, and GPx [32]. As a primer antioxidant enzyme, SOD (a key antioxidant in living cells) is transform a superoxide reactive radical into hydrogen peroxidase.

The conversion of hydrogen peroxidase to water and molecular oxygen is carried out by CAT [32]. But hydrogen peroxide leads to the formation of hydroxyl radical owing to the lowering of CAT in the tissue. GPx prevents TBARS from ROS using the thiol-lowering power of glutathione. Although MTX is widely used in the clinic, there are many side effects of its in addition to organ toxicity. Previous scientific studies have shown that MTX directly initiates oxidative damage of membrane lipids in organs such as the liver and renal, by rising the malondialdehyde (MDA) level one of the pathological biomarkers as lipid peroxidation end product. Also, another study demonstrated that MTX leads to an increment in MDA level and reduce in SOD and CAT activities in rats. They have reported with numerical findings that oxidative injury leads to organ damage stimulated by an antineoplastic agent [33]. Some antioxidant enzymes such as CAT and GPx catalyze hydrogen peroxide [39]. GST induces the connection of GSH to xenobiotic agents to eliminate their toxic effects. Activities of GST and GPx are relying on a continuous providing of GSH. Hence, an important lowering in liver CAT and GPx activities in MTX-given rats can be connected with ROS generation causing a depletion of antioxidants to provide, which finally concludes in oxidative injury to the cell membrane, as is signaled by increasing MDA levels [34]. The previous investigation suggested MTX-administered organ injury, and the results of the study showed increased myeloperoxidase (MPO) levels, due to MTX treatment. The aforementioned results are in the same direction as the works we offer. Our research showed that levels of MPO and MDA have risen in the MTX-treated group to the control group, and the SOD, CAT, and GPx activities were elevated in the MTX-treated group compared to the control group. CAT, SOD, and GPx, activities increased in BAR +MTX group, but MDA and MPO levels were reduced in hepatic samples in previous different studies [36]. These data may be based on the abatement of the oxidation of lipids owing to the strong antioxidant efficiency of BAR. Also, our results are in the same direction as the findings of Tanyeli et al. [19], In their hepatotoxicity cisplatin-induced study, it was determined the organ-protective and antioxidant effects of barbaloin.

The proportion of abnormal liver enzymes had been seen differently broadly among researchers. A recent review indicated that increased transaminases (levels greater than twice the upper limit of normal values) were detected in a particular section of patients used chronically with low-dose methotrexate [38,39]. Also, in our study, induced by MTX evident liver tissue injury as seen by biochemical changes in serum. These changes involved an increase in hepatic injury biomarkers (AST and ALT) (figure 5). These alterations are harmonious with those results of Abdellatief et al. [35], Amal Ahmet et al. [40], and Hafez et al. [4]. Biochemical parameters of hepatic tissue injury (ALT and AST) were increased only in the MTX-treated group. Treatment of barbaloin restored serum levels of tissue-injury biological parameters such as ALT and AST.

Conclusion

As a result, barbaloin has an important curable effect against the hepatotoxicity induced by methotrexate therapy.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

This study was approved by the Ataturk University Animal Experiments Local Ethics Committee (Approval no: 75296309-050.01.04-E.2100036416).

References

1. Stephens C, Andrade RJ, Lucena MI. Mechanisms of drug-induced liver injury. Curr Opin Allergy Cl. 2014;14:286-92.

2. Kumar V, Kalita J, Misra UK, Bora HK. A study of dose response and organ susceptibility of copper toxicity in a rat model. J Trace Elem Med Bio. 2015;29:269-74.

3. Akbulut S, Elbe H, Eris C, et al. Cytoprotective effects of amifostine, ascorbic acid and N-acetylcysteine against methotrexate-induced hepatotoxicity in rats. World J Gastroenterol. 2014;20:10158-65.

4. Hafez HM, Ibrahim MA, Ibrahim SA, et al. Potential protective effect of etanercept and aminoguanidine in methotrexate-induced hepatotoxicity and nephrotoxicity in rats. Eur J Pharmacol. 2015;5:1-12.

5. Mukherjee S, Ghosh S, Choudhury S, et al. Chattopadhyay S: Pomegranate reverses methotrexate-induced oxidative stress and apoptosis in hepatocytes by modulating Nrf2-NF-kappa B pathways. J. Nutr. Biochem. 2013;24:2040-50.

6. Koksal E, Bursal E, Gulcin İ, et al. Antioxidant activity and polyphenol content of Turkish thyme (Thymus vulgaris) monitored by LC-MS/MS. Int. J. Food Prop. 2017;20:514-25.

7. Hamad HO, Alma MH, Gulcin İ, Yılmaz MA, et al. Evaluation of phenolic contents and bioactivity of root and nutgall extracts from Iraqian Quercus infectoria Olivier. Rec Nat Prod. 2017;11:205-10.

8. Tohma H, Köksal E, Kılıç Ö, et al. RP-HPLC/MS/MS analysis of the phenolic compounds, antioxidant and antimicrobial activities of Salvia L. species. Antioxidants. 2016;5:38.

9. Bae JH, Park YJ, Namiesnik J, et al. Effects of artificial lighting on bioactivity of sweet red pepper (Capsicum annuum L.). Int J Food Sci Tech. 2016;51:1378-85.

10. Topal F, Topal M, Gocer H, et al. Antioxidant activity of taxifolin: An activity-structure relationship. J Enzyme Inhib Med Chem. 2016;31:674-83.

11. Topal M, Gocer H, Topal F, et al. Antioxidant, antiradical and anticholinergic properties of cynarin purified from the illyrian thistle (Onopordum illyricum L.). J Enzyme Inhib Med Chem. 2016: 31: 266-275.

12. Polat Köse L, Gülçin İ, Gören AC, et al. LC-MS/MS analysis, antioxidant and anticholinergic properties of galanga (Alpinia officinarum Hance) rhizomes. Ind Crops Prod. 2016;74:712–21.

13. Öztaşkın N, Çetinkaya Y, Taslimi P, et al. Antioxidant and acetylcholinesterase inhibition properties of novel bromophenol derivatives. Bioorg Chem. 2015;60:49-57.

14. Gülçin İ. Antioxidant activity of food constituents-An overview. Arch Toxicol. 2021;86:345-91.

15. Gouveia LDV, Cardoso CA, De Oliveira GMM, et al. Effects of the Intake of Sesame Seeds (Sesamum indicum L.) and Derivatives on Oxidative Stress: A Systematic Review. J Med Food. 2016;19:337-45.

16. Moghadam AR, Tutunchi S, Namvaran Abbas Abad A, et al. Pre-administration of turmeric prevents methotrexate-induced liver toxicity and oxidative stress. BMC Complem Altern M. 2015;15:246.

17. Hemeida RA, Mohafez OM. Curcumin attenuates methotraxate-induced hepatic oxidative damage in rats. J Egypt Nat Canc Ins. 2008;20:141-814.

18. Vardi N, Parlakpinar H, Cetin A, et al. Protective effect of beta-carotene on methotrexate-induced oxidative liver damage. Toxicol Pathol. 2010;38:592-7.

19. Tanyeli A, Guler MC, Eraslan E, et al. Barbaloin attenuates ischemia reperfusion-induced oxidative renal injury via antioxidant and anti-inflamatory effects. Med Science. 2020;9:246-50.

20. Uchiyama M, Mihara M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;34:271-8,

21. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Bio Chem. 1951;193:265-75.

22. Sun Y, Oberley LW, Li Y. A Simple Method for Clinical Assay of Superoxide-Dismutase. Clin Chem. 1988;34:497-500.

23. Aebi H. Catalase in vitro. Method Enzymol. 1984;105:121-6.

24. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:158-69.

25. Wei HC, Frenkel K. Relationship of oxidative events and DNA oxidation in sencar mice to in vivo promoting activity of phorbol ester-type tumor promoters. Carcinogen. 1993;14:1195-201.

26. Kovacevic A, Hammer A, Stadelmeyer E, et al. Expression of serum amyloid A transcripts in human bone tissues, differentiated osteoblast-like stem cells and human osteosarcoma cell lines. J Cell Biochem. 2008;103:994-1004.

27. Waseem M, Pandey P, Tomar B, et al. Ameliorative action of curcumin in cisplatin-mediated hepatotoxicity: an in vivo study in wistar rats. Arch Med Res. 2014;45:462-68.

28. Gülçin İ, Topal F, Çakmakçı R, et al. Pomological features, nutritional quality, polyphenol content analysis and antioxidant properties of domesticated and three wild ecotype forms of raspberries (Rubus idaeus L.). J Food Sci. 2011;76:585-93.

29. Babiak RM, Campello AP, Carnieri EG, et al. Methotrexate pentose cycle and oxidative stress. Cell Biochem. Funct. 1998;16:283-93.

30. Gulcin I. Antioxidant activity of eugenol: a structure-activity relationship study. J Med Food. 2011;14: 975-85.

31. Gulcin I, Mshvildadze V, Gepdiremen AA, et al. Antioxidant activity of saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F. Planta Med. 2004;70:561-3.

32. Moselhy SS, Ali HKH. Hepatoprotective effect of Cinnamon extracts against carbon tetrachloride induced oxidative stress and liver injury in rats. Biol Res. 2009;42:93-8.

33. Köksal E, Ağgül AG, Bursal E, et al. Purification and characterization of peroxidase from sweet gourd (Cucurbita Moschata Lam. Poiret). Int J Food Prop. 2012;15:1110-9.

34. Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol. 2006;141:312-22.

35. Abdellatief SA, Galal AA, Farouk SM, et al. Ameliorative effect of parsley oil on cisplatin-induced hepato-cardiotoxicity: A biochemical, histopathological, and immunohistochemical study. Biomed Pharmacother. 2017;86:482-91.

36. Cetinkaya A, Kurutas EB, Bulbuloglu E, Kantarceken B. The effects of n-acetylcysteine on methotrexate-induced oxidative renal damage in rats. Nephrol Dial Transpl. 2007;22:284-5.

37. Kahraman H, Kurutas E, Tokur M, et al. Protective Effects of Erythropoietin and N-Acetylcysteine on Methotrexate-Induced Lung Injury in Rats. Balk Med J. 2013;30:99-104.

38. Conway R, Low C, Coughlan RJ, et al. Risk of liver injury among methotrexate users: A meta-analysis of randomised controlled trials. Semin Arthritis Rheu. 2015;45:156-62.

39. Salliot C, Van Der Heijde D. Long-term safety of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic literature research. Ann Rheum Dis. 2009;68:1100-4.

40. Ahmed AA, Selim MA, El Sayed NM. Alpha-Lipoic acid ameliorates oral mucositis and oxidative stress induced by methotrexate in rats. Histological and immunohistochemical study. Life Sci. 2017;171:51-9.