Original
Nuclear Medicine Review 2018, 21, 1: 8–13
DOI: 10.5603/NMR.a2018.0006

ISSN 1506–9680

VIA MEDICA

The use of ^{99m}Tc-phytate for assessment the protective effect of vitamin E against hepatotoxicity induced by methotrexat in rat

Hossein Amirfakhrian[1], Seyed Mohammad Abedi[2], Hossein Sadeghi[1], Soheil Azizi[3], Seyed Jalal Hosseinimehr[1]
[1]Department of Radiopharmacy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[2]Department of Radiology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[3]Department of Laboratory Medicine, Faculty of Allied Medical Sciences, Mazandaran University of Medical Sciences, Sari, Iran

[Received 11 I 2017; Accepted 30 XI 2017]

Abstract

Hepatotoxicity is one of the most common side effects of methotrexate (MTX) therapy in patients. The aim of this study was to evaluate the protective effect of vitamin E against MTX-induced hepatotoxicity using ^{99m}Tc-phytate as a radiopharmaceutical agent in animals. Rats were divided into five groups as follows: control, solvent, Vit E (100 mg/kg), MTX (20 mg/kg) and Vit E + MTX. Animals were intraperitoneally injected with Vit E for 17 days before MTX injection and continued for 4 days. ^{99m}Tc-phytate was injected into the tail of rats after the 21 days of Vit E administration. Percentage of the injected dose per gram of liver and spleen tissues (%ID/g) was calculated in treated rats. Liver imaging was obtained with gamma camera. In other experiment, liver of treated rats was assessed for histopathology. ^{99m}Tc-phytate uptake (%ID/g) of livers in control, solvent, Vit E, MTX and Vit E + MTX groups were 8.99% ± 1.37, 8.53% ± 2.91, 8.65% ± 3.84, 3.22% ± 1.09 and 8.38% ± 2.68. Vit E administration resulted in a significant increase of the level of %ID/g in MTX-injected animal. Vit E pre-treatment improved the shape of liver in MTX-treated rat which was seen abnormal view in planar imaging. Histophatological examinations approved the protective effect of Vit E against MTX-induced hepatotoxicity in rats. The results of this study show that Vit E significantly attenuates the MTX-induced hepatotoxicity in rats, and ^{99m}Tc-phytate is an acceptable radiopharmaceutical agent for assessment of liver and spleen damages in the animal model.

KEY words: Vitamin E, liver, ^{99m}Tc-phytate, hepatotoxicity, hepatoprotective

Nucl Med Rev 2018; 21, 1: 8–13

Introduction

Methotrexate (MTX) is a folic acid antagonist which has been widely used as an effective anticancer agent in chemotherapy regimen [1]. It has been prescribed extensively as a cytotoxic agent in the treatment of leukemia, breast cancer, testicular tumors and other malignancies. It is used in psoriasis and rheumatoid arthritis that are inflammatory related diseases [2–4]. MTX causes some toxic effects including nausea, vomiting, diarrhea, alopecia, stomatitis, bone marrow depression and hepatotoxicity in patients [5]. MTX treatment increases biomarkers associated with liver injury and are involving in hepatotoxicity [6]. MTX inhibits enzymes corresponding in production of the main sources of nicotine amide adenine dinucleotide (NADPH) resulting in intracellular decreases of NADPH [7]. Following NADPH decreasing, glutathione decreases and hepatocytes become vulnerable against reactive oxygen species (ROS). Enhancement of ROS level causes oxidative stress in cell [8]. Hepatotoxicity and production of ROS are main side effects induced by MTX [9]. Then it has been advised to prevent MTX-induced hepatotoxicity, it is using concomitant with antioxidants [10]. Several protective agents such as penthoxyphyllin, alpha lipoic acid and beta carotenes have been investigated in different studies for prevention of MTX-caused hepatotoxicity [10, 11]. Vitamin E is considered a lipid soluble vitamin and an important antioxidant. This vitamin is able to neutralize peroxide and free radicals. Vitamin E protects cellular membrane against oxidative damages [12]. Vitamin E, as a free radical scavenger, protects cellular organelles against harmful effects of free radicals [13].

^{99m}Tc-phytate colloid is a radiopharmaceutical agent that has been used for functional imaging of liver and spleen in nuclear medicine. Since 1973, ^{99m}Tc-phytate has been used as an imaging

Correspondence to: Seyed Jalal Hosseinimehr, Department of Radiopharmacy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran, e-mail: sjhosseinim@yahoo.com

radiotracer for liver and spleen assessments. Most of the colloid particles are captured by the Kupffer cells in the liver which is well imaged in nuclear medicine practice [14, 15]. Radionuclide distribution in the liver has a great relevance with chronic hepatic disease severity, histological fibrosis severity and hepatic functions. So, quantitative evaluation of ^{99m}Tc-phytate uptake in liver could be used as a hepatic function tool [16].

The purpose of this research was to evaluate the protective effect of vitamin E on hepatic and spleen injuries caused by MTX using quantitative ^{99m}Tc-phytate uptake measurements and planar imaging and then radiopharmaceutical findings were compared with pathological data in rat.

Material and methods

Animal

Wistar rats weighing 180 ± 220 g were purchased from the Pasteur Institute (Amol, Iran). Rats were housed in a good condition in the university animal house and given standard mouse pellet and water ad Libitum. Animals were kept under controlled lighting condition (light: dark, 12:12 h) and temperature (22 ± 1°C). Animal experimental and ethical issues were approved by Research Committee of Mazandaran University of Medical Sciences, Sari, Iran (ID# 1969).

Chemicals

MTX was purchased from Myian (2 mg/2 mL vial, France). Vitamin E (Vit E), as injection form in olive oil, was from Osvaeh Pharmaceutical Co. (Iran). All other chemicals were obtained from Merck Company (Germany).

Experimental group and treatment schedules

Twenty animals were randomly divided into 5 groups (four rats each group):

- Group 1: control; the rats were administered daily intraperitoneal (ip) of normal saline for 21 days;
- Group 2: solvent; the rats were administered daily intraperitoneal (ip) of olive oil for 21 days;
- Groups 3: Vit E; the rats were treated with Vit E (100 mg kg^{-1} bw) for 21 days;
- Group 4: MTX; the rats were administered a single dose of MTX ip injections (20 mg kg^{-1} body weight (bw)) at 17 days after olive oil treatment and 4 days before killing;
- Groups 5: Vit E+ MTX; rats were injected with Vit E (100 mg kg^{-1} bw) at 21 days before single dose of MTX treatment and continued for 4 days.

Radiopharmaceutical assessment

^{99m}Tc, as sodium pertechnetate, was eluted from a ^{99}Mo/^{99m}Tc generator (IEOI, Iran) just before the radiolabelling procedure. Phytate (IEOI, Iran) was used as a freeze-dried commercial kit. ^{99m}Tc-phytate was prepared by adding 740 MBq of ^{99m}Tc-pertechnetate to 3.5 mL of saline to the kit. Radiolabelling was performed according to manufacture guideline. After slowly shaking, it is kept at room temperature for 15 minute. ^{99m}Tc-phytate (0.1 mL) was injected through the tail vein. After deep anesthesia (by ketamine/xylazine), rats were killed and the livers and spleens were removed by dissection 2 hours after the radiopharmaceutical injection. Livers and spleens were weighed in pre-weighed containers. Radioactivity in each organ samples was counted using a gamma counter (Delshid, Iran). The percentage of injected dose per gram of tissue (%ID/g) was calculated by dividing the radioactivity count per minute in each tissue by total count injected and the mass of the organ.

Scintigraphic imaging with ^{99m}Tc-phytate

Planar imaging studies were performed on anesthetized rats to obtain a visual confirmation of the liver uptakes and shapes. Images were acquired at 2 hours postinjection of ^{99m}Tc-phytate using an E-CAM dual head (Siemens Medical Solutions, Hoffman Estates, IL) equipped with a low energy high-resolution collimator.

Histopathological assessment

After fixation the liver tissue in 10% formalin, samples were processed according to standard histological techniques and embedded in paraffin blocks. Paraffin blocks were sectioned using a rotary microtome into 3 μm thick sections placed on glass slides and fixed for histological staining. Staining was performed using hematoxylin. Liver tissue was evaluated on hepatic paranchyme showing morphological changes. The tissue sections were assessed by blinded pathologist. The average of ten microscopic fields was recorded by him for each specimen. Histopathological criteria and the morphological parameters were defined semi-quantitatively according to modified scoring system [17, 18] which performed as follow:

(-), (+-), (+), (++), and (+++) is indicating: no or absent, few or $<$ 1 single altered foci into microscopic HPF (High Power Field $\times$400), mild or between 1 $\leq$ 2 altered foci in 10 microscopic HPF, moderate or between 2–3 altered foci in to microscopic HPF, and severe or $>$ 3 altered foci in to microscopic HPF, respectively.

Statistical analysis

The data values are presented as means ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), as well as post hoc Dunnett's Multiple Comparison Test. P value $<$ 0.05 was considered as significant and highly significant, respectively (Prism Software, USA).

Results

Quantitation of liver and spleen uptakes of ^{99m}Tc-phytate

The percentage of the injected dose per gram of ^{99m}Tc-phytate in liver of control, solvent, Vit E, MTX, and Vit E + MTX groups were 8.99% ± 1.37, 8.53% ± 2.91, 8.65% ± 3.84, 3.22% ± 1.09 and 8.38% ± 2.68 (Figure 1A). MTX injection reduced ^{99m}Tc-phytate uptake in liver as compared with other groups as control, solvent and Vit E. It was observed significant difference between MTX group and other groups in ID/g% values of livers (P $<$ 0.05). Vit E pre-treatment significantly increased the ^{99m}Tc-phytate uptake in the livers of rats injected by MTX. The mean of ID/g% value in liver was significantly higher in Vit E + MTX group as compared with MTX treatment alone group (P $<$ 0.05). The ID/g% of ^{99m}Tc-phytate in spleen of control, solvent, Vit E, MTX, and Vit E + MTX groups were 4.00% ± 1.07, 5.19% ± 1.34, 4.40% ± 2.11, 1.86% ± 0.49 and 2.94% ± 0.98 (Figure 1B). It was observed a significant difference between MTX group with solvent and Vit E groups in ID/g% values (P $<$ 0.05). The ^{99m}Tc-phytate spleen uptake was about 2.7

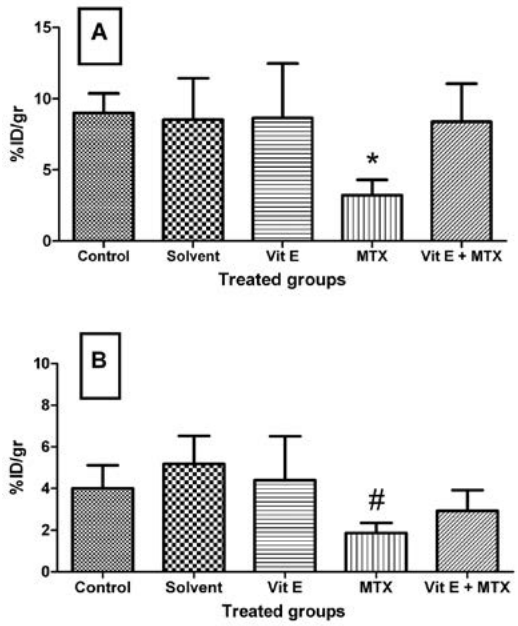

Figure 1. The percentage of the injected ^{99m}Tc-phytate dose per gram of liver (**A**) and spleen (**B**) tissues (% ID/g) in control, solvent (olive oil), vitamin E (100 mg/kg), methotrexate (MTX), vitamin E + methotrexate (Vit E + MTX) groups are shown (n = 4). *Comparison of all groups with MTX groups $P < 0.05$, non-significant comparison of control, solvent, Vit E and Vit E + MTX between groups. # Comparison of solvent and Vit E with MTX groups $P < 0.05$, non-significant comparison of control and Vit E + MTX with MTX

fold less in MTX treatment group as compared to solvent group ($P < 0.05$). However Vit E pre-treatment increased the ^{99m}Tc-phytate uptake in the spleens of rats injected by MTX, the mean of ID/g% value in spleen was statistically non significantly higher in Vit E + MTX group as compared with MTX treatment alone group.

Gamma camera imaging

The whole body images of treated rats are presented in Figure 2. The hepatic uptakes of ^{99m}Tc-phytate were homogeneous in control, solvent and Vit E treated rats. The shape of liver was abnormal in MTX-injected rat. The uptake of ^{99m}Tc-phytate was concentrated as a circle or round shape. It is interesting that the Vit E pre-treatment improved the shape of liver in planar image.

Histopathological assessment

The impaired hepatic caused by MTX was further confirmed by histological examination of liver (Fig. 3). As shown in Figure 3, liver sections from control, solvent and Vit E in rats showed normality whereas liver in animals treated with MTX revealed a marked liver necrosis and degeneration, extensive epithelial vacuolization, swelling and dilation (Fig. 3). The hepatic damage was less severe in the rat treated with Vit E + MTX when compared with MTX-treated alone rat (Fig. 3). The histopathological alteration semi-quantitative results of some injury related parameters are shown in Table 1. Histological evaluations of the liver sections of animals from control, solvent and Vit E groups showed normal structure, architectural integrity and regenerative nodules. The animals from MTX group

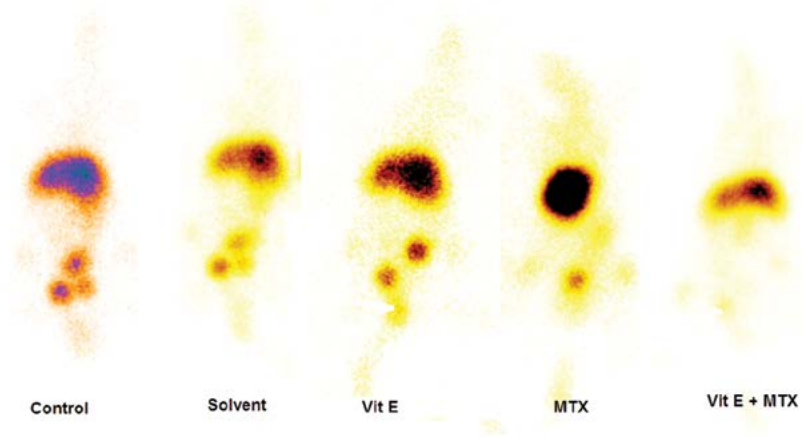

Figure 2. Whole body images of rats were injected ^{99m}Tc-phytate. Rats are as control, solvent, Vit E, methotrexate (MTX), Vit E + MTX. The uptakes of ^{99m}Tc-phytat in control, solvent and Vit E and also Vit E + MTX are homogenous while scintigraphic imaging shows an abnormal shape of liver in MTX-treated rat

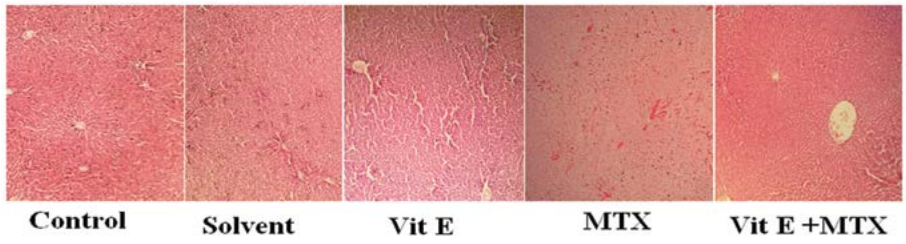

Figure 3. Histological examination of rats treated with methotroxate (MTX) and/or vitamin E (Vit E). The liver of control, solvent and Vit E treated rats show normal. Liver of rat administered with MTX at a single dose of 20 mg/Kg exhibited a distinct histological difference when compared with control, these liver shows large numbers of cells with degeneration. Improved cellular degeneration was seen in rat treated with Vit E at dose 100 mg/Kg and MTX

Table 1. The effect of vitamin E (Vit E) against hepatotoxicity induced by methotrexate (MTX)

	Criteria				
Group	Control	Solvent	Vit E	MTX	Vit E + MTX
Change in architecture	-	-	-	+	-
Congestion	-	+,-	+,-	+++	++
Dilation	-	+,-	+,-	+++	+
PMNS	-	+,-	+	+++	+
Mononuclears	-	-	+,-	++	+
Eosinophills	-	-	-	++	-
Necrosis	-	-	+,-	+	+,-
Apoptosis	-	-	-	++	+
Cytoplasmic	-	-	+,-	++	+
Hydropic degeneration	-	-	+,-	++	+
Activated kupffer cell	-	-	+,-	++	+
Fatty change macrovesicular steatosis	-	++	+,-	-	-
Uncertain cellular limit	-	+,-	+,-	++	+,-
Tronbosis	-	-	-	++	+
Endothelial cells injuries	-		-	++	+,-

(-), (+,-), (+), (++), and (+++) is indicating: no or absent, few or $<$ 1 single altered foci in to microscopic HPF (High Power Field *400), mild or between 1 $\leq$ 2 altered foci in 10 microscopic HPF, moderate or between 2–3 altered foci in to 10 microscopic HPF, and severe or $>$ 3 altered foci in to 10 microscopic HPF, respectively

exhibited histopathological alterations, with variations in the number of pathological criteria such as of congestion, dilatation, apoptotic and cytoplasmic and endothelial cell injury (Table 1). Vit E + MTX group showed preserved liver architecture with markedly reduction in number of pathological alterations.

Discussion

It is important to recognize and understand the side effect of chemotherapy on the liver, since this is a major organ which is responsible for drug, endogenous and exogenous substances clearance. Since some chemotherapeutic agents such as MTX may cause liver damage, the evaluation of possible hepatotoxicity is important in healthcare. In this study, hepatoprotective effect of Vit E after a single injection of MTX was evaluated in rats. MTX induced significantly live damage that was prevented by Vit E treatment. The hepatotoxicity was evaluated using ^{99m}Tc-phytate that is used as a radiopharmaceutical agent for assessment of hepatic and spleen diseases in patients. This protective effect was confirmed with histopathology. This is the first time study to investigate using MTX in treatment with Vit. E in animals that was verified with a radionuclide method using ^{99m}Tc-phytate as a radioactive colloid radiopharmaceutical. Hepatotoxicity is a common side effect caused by MTX in patients [19, 20]. Elevated ROS and induced pro-inflammatory process are main suggested mechanisms involved in hepatotoxicity induced by MTX [21]. Several compounds have been reported to have protective effect against hepatotoxicity induced by MTX. These compounds acted mainly through antioxidant and anti-inflammatory effects [22–24]. Our findings showed that MTX significantly reduced the liver uptake of ^{99m}Tc-phytate about 2.5-fold less level than control rats. The present results show that MTX causes Kupffer cell malfunction in the liver and these damaged cells unable to uptake normally this radiopharmaceutical agent. Also the reduction in uptake of ^{99m}Tc-phytate was observed in spleen of MTX-treated rats. The reticuloendothelial system (RES) plays an important role in the biological defense system. Kupffer cells are the main constituent of the RES in the liver and spleen, and when their function is impaired post drug treatment the complications may occur [25, 26]. ^{99m}Tc-phytate is used for imaging areas of functional reticuloendothelial cells in the liver, spleen and bone marrow. It is a useful radiopharmaceutical agent in the diagnosis, localization and evaluation of liver and spleen pathology. In the blood, phytic acid binds with calcium ions to form colloidal particles which are captured by the RES, principally by Kupffer cells in the liver. In imaging purpose, ^{99m}Tc-phytate reacts *in vivo* with calcium ions in the blood and forms ^{99m}Tc-Sn-Ca-phytate that is rapidly cleared by the RES from the blood. Uptake of the radioactive colloid by RES is dependent upon both the relative blood flow rates and the functional capacity of the phagocytic cells [27]. In a study, the side effect of ionizing radiation on the liver in rat was evaluated using gamma camera (^{99m}Tc-phytate) imaging and MRI [28]. Exposure to ionizing radiation (4 Gy) markedly induced liver injury that was visualized by gamma scintigraphy with changing in the shape of the liver and the relative proportions of the volumes of its right and left portions that these findings were confirmed by MRI and pathology. Both techniques proved that the reticuloendothelial cells were damaged by ionizing radiation, which might result in malfunction of the Kupffer cells and or a decrease in their number [28]. Histological findings of MTX-induced hepatotoxicity are including macrovesicular steatosis, hepatocellular necrosis and inflammation, edema, fibrosis, morphological abnormalities and development of cirrhosis [29–31]. Chemotherapeutic agents caused drug induced acute hepatitis and hepatomegaly, decreased liver enhancement and widening of periportal space due to edema which are nonspecific imaging findings of acute hepatitis [32]. In our study an abnormal shape of liver in MTX-treated animal that attributed liver pathologic abnormalities was observed.

In this study, Vit E administration increased the liver uptake of ^{99m}Tc-phytate in rats injected with MTX, and the level of radioactivity uptake was close to normal range. It was interesting that the shape of liver was changed in MTX-injected rat and liver was an abnormal shape. While the shape of liver in Vit E + MTX treated rat was similar to control rat. It was a confirmation in liver damages assessment between quantitation of liver uptake of ^{99m}Tc-phytate and liver image. Pathological examination confirmed that MTX significantly caused hepatotoxicity in rats that this liver damage was prevented by Vit E treatment. Vit E exhibited hepatoprotective effect in rats that co-injected with MTX. Vit E has free radical scavenging, increasing endogenous antioxidant and anti-inflammatory effects [33, 34]. These mechanisms are suggested to contribute in reduction of deleterious effects of MTX in liver and spleen by Vit E.

Conclusion

In this study, it was demonstrated the Vit E was effective in the prevention of hepatotoxicity induced by MTX in animal. Our results showed that quantization of injected dose per gram of ^{99m}Tc-phytate in liver and spleen as well as liver image to be acceptable techniques for assessment of liver and spleen damages and/or their tissues protective effects in animal model.

Conflict of interest statement

The authors declared no potential conflict of interest with respect to the authorship, and/or publication of this study.

Acknowledgments

This study was supported by a grant from Mazandaran University of Medical Sciences, Sari, Iran (number 1969).

References

1. Peters GJ, van der Wilt CL, van Moorsel CJ, et al. Basis for effective combination cancer chemotherapy with antimetabolites. Pharmacol Ther. 2000; 87(2-3): 227–253, doi: 10.1016/s0163-7258(00)00086-3, indexed in Pubmed: 11008002.
2. Choudhury RC, Ghosh SK, Palo AK. Cytogenetic toxicity of methotrexate in mouse bone marrow. Environ Toxicol Pharmacol. 2000; 8(3): 191–196, doi: 10.1016/s1382-6689(00)00041-7, indexed in Pubmed: 10925072.
3. Jensen SB, Mouridsen HT, Reibel J, et al. Adjuvant chemotherapy in breast cancer patients induces temporary salivary gland hypofunction. Oral Oncol. 2008; 44(2): 162–173, doi: 10.1016/j.oraloncology.2007.01.015, indexed in Pubmed: 17588802.
4. Sener G, Ekşioğlu-Demiralp E, Cetiner M, et al. L-Carnitine ameliorates methotrexate-induced oxidative organ injury and inhibits leukocyte death. Cell Biol Toxicol. 2006; 22(1): 47–60, doi: 10.1007/s10565-006-0025-0, indexed in Pubmed: 16463019.
5. Rang HP, Dale MM, Ritter JM, Flower RF, Henderson G. Rang & Dale's Pharmacology. 5th. Churchill Livingston, London 2003: 490–502.
6. Thomas JA, Aithal GP. Monitoring liver function during methotrexate therapy for psoriasis: are routine biopsies really necessary? Am J Clin Dermatol. 2005; 6(6): 357–363, doi: 10.2165/00128071-200506060-00003, indexed in Pubmed: 16343024.
7. Caetano NN, Campello AP, Carnieri EG, et al. Effect of methotrexate (MTX) on NAD(P)+ dehydrogenases of HeLa cells: malic enzyme, 2-oxoglutarate and isocitrate dehydrogenases. Cell Biochem Funct. 1997; 15(4): 259–264, doi: 10.1002/(SICI)1099-0844(199712)15:4<259::AID-CBF749>3.0.CO;2-D, indexed in Pubmed: 9415972.
8. Uraz S, Tahan V, Aygun C, et al. Role of ursodeoxycholic acid in prevention of methotrexate-induced liver toxicity. Dig Dis Sci. 2008; 53(4): 1071–1077, doi: 10.1007/s10620-007-9949-3, indexed in Pubmed: 17934844.
9. Babiak RM, Campello AP, Carnieri EG, et al. Methotrexate: pentose cycle and oxidative stress. Cell Biochem Funct. 1998; 16(4): 283–293, doi: 10.1002/(SICI)1099-0844(1998120)16:4<283::AID-CBF801>3.0.CO;2-E, indexed in Pubmed: 9857491.
10. 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. Environ Toxicol Pharmacol. 2015; 39(3): 1122–1131, doi: 10.1016/j.etap.2015.04.003, indexed in Pubmed: 25912852.
11. Vardi N, Parlakpinar H, Cetin A, et al. Protective effect of beta-carotene on methotrexate-induced oxidative liver damage. Toxicol Pathol. 2010; 38(4): 592–597, doi: 10.1177/0192623310367806, indexed in Pubmed: 20448084.
12. Gulec M, Gurel A, Armutcu F. Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. Mol Cell Biochem. 2006; 290(1-2): 61–67, doi: 10.1007/s11010-006-9165-z, indexed in Pubmed: 16937016.
13. Ozdil S, Bolkent S, Yanardag R, et al. Protective effects of ascorbic acid, dl-alpha-tocopherol acetate, and sodium selenate on ethanol-induced liver damage of rats. Biol Trace Elem Res. 2004; 97(2): 149–162, doi: 10.1385/BTER:97:2:149, indexed in Pubmed: 14985625.
14. Groshar D, Slobodin G, Zuckerman E. Quantitation of liver and spleen uptake of (99m)Tc-phytate colloid using SPECT: detection of liver cirrhosis. J Nucl Med. 2002; 43(3): 312–317, indexed in Pubmed: 11884489.
15. Hoefs JC, Wang F, Kanel G, et al. The liver-spleen scan as a quantitative liver function test: correlation with liver severity at peritoneoscopy. Hepatology. 1995; 22(4 Pt 1): 1113–1121, doi: 10.1002/hep.1840220415, indexed in Pubmed: 7557859.
16. Picard D, Infante-Rivard C, Villeneuve JP, et al. Extrahepatic uptake of technetium-99m-phytate: a prognostic index in patients with cirrhosis. J Nucl Med. 1990; 31(4): 436–440, indexed in Pubmed: 2324819.
17. Gonzalez P, Humeres P, Arroyo A, et al. Liver uptake with phytate colloid in normal subjects and patients with cirrhosis: a possible alternative to extrahepatic measurements—reply. J Nucl Med. 1991; 32(7): 1467–1468, indexed in Pubmed: 2066812.
18. Huet PM, Chartrand R, Marleau D. Extrahepatic uptake of 99mTc-phytate: its mechanism and significance in chronic liver disease. Gastroenterology. 1980; 78(1): 76–80, indexed in Pubmed: 7350039.
19. Gao Li, Yang F, Ren C, et al. Diagnosis of cirrhotic portal hypertension and compensatory circulation using transsplenic portal scintigraphy with (99m)Tc-phytate. J Nucl Med. 2010; 51(1): 52–56, doi: 10.2967/jnumed.109.067983, indexed in Pubmed: 20008994.
20. Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. Hepatology. 2000; 31(1): 241–246, doi: 10.1002/hep.510310136, indexed in Pubmed: 10613753.
21. Zhang C, Wang Y, Chen H, et al. Protective effect of the herbal medicine Gan-fu-kang against carbon tetrachloride-induced liver fibrosis in rats. Mol Med Rep. 2013; 8(3): 954–962, doi: 10.3892/mmr.2013.1587, indexed in Pubmed: 23857550.
22. Abolmaali SS, Tamaddon AM, Dinarvand R. A review of therapeutic challenges and achievements of methotrexate delivery systems for treatment of cancer and rheumatoid arthritis. Cancer Chemother Pharmacol. 2013; 71(5): 1115–1130, doi: 10.1007/s00280-012-2062-0, indexed in Pubmed: 23292116.
23. Hazlewood GS, Barnabe C, Tomlinson G, et al. Methotrexate monotherapy and methotrexate combination therapy with traditional and biologic disease modifying anti-rheumatic drugs for rheumatoid arthritis: A network

meta-analysis. Cochrane Database Syst Rev. 2016; 353(8): CD010227, doi: 10.1002/14651858.cd010227.pub2, indexed in Pubmed: 27102806.
24. Puig L. Methotrexate: new therapeutic approaches. Actas Dermosifiliogr. 2014; 105(6): 583–589, doi: 10.1016/j.ad.2012.11.017, indexed in Pubmed: 23434058.
25. Sotoudehmanesh R, Anvari B, Akhlaghi M, et al. Methotrexate hepatotoxicity in patients with rheumatoid arthritis. Middle East J Dig Dis. 2010; 2(2): 104–109, indexed in Pubmed: 25197521.
26. Barker J, Horn EJ, Lebwohl M, et al. International Psoriasis Council. Assessment and management of methotrexate hepatotoxicity in psoriasis patients: report from a consensus conference to evaluate current practice and identify key questions toward optimizing methotrexate use in the clinic. J Eur Acad Dermatol Venereol. 2011; 25(7): 758–764, doi: 10.1111/j.1468-3083.2010.03932.x, indexed in Pubmed: 21198946.
27. Albrecht K, Müller-Ladner U. Side effects and management of side effects of methotrexate in rheumatoid arthritis. Clin Exp Rheumatol. 2010; 28(5 Suppl 61): S95–101, indexed in Pubmed: 21044440.
28. Mukherjee S, Ghosh S, Choudhury S, et al. Pomegranate reverses methotrexate-induced oxidative stress and apoptosis in hepatocytes by modulating Nrf2-NF-κB pathways. J Nutr Biochem. 2013; 24(12): 2040–2050, doi: 10.1016/j.jnutbio.2013.07.005, indexed in Pubmed: 24231097.
29. Dalaklioglu S, Genc GE, Aksoy NH, et al. Resveratrol ameliorates methotrexate-induced hepatotoxicity in rats via inhibition of lipid peroxidation. Hum Exp Toxicol. 2013; 32(6): 662–671, doi: 10.1177/0960327112468178, indexed in Pubmed: 23424212.
30. Ali N, Rashid S, Nafees S, et al. Beneficial effects of Chrysin against Methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. Mol Cell Biochem. 2014; 385(1-2): 215–223, doi: 10.1007/s11010-013-1830-4, indexed in Pubmed: 24154663.
31. Cure E, Kirbas A, Tumkaya L, et al. Protective effect of infliximab on methotrexate-induced liver injury in rats: unexpected drug interaction. J Cancer Res Ther. 2015; 11(1): 164–169, doi: 10.4103/0973-1482.140809, indexed in Pubmed: 25879356.
32. Hamazaki K, Sato S, Yunoki M, et al. Kupffer cell function in chronic liver injury and after partial hepatectomy. Res Exp Med (Berl). 1994; 194(4): 237–246, doi: 10.1007/bf02576385, indexed in Pubmed: 7800933.
33. Watanabe T, Kondo M, Hirasa M, et al. Scintigraphic study of regenerative nodules due to fulminant hepatic failure. J Gastroenterol. 2003; 38(7): 695–699, doi: 10.1007/s00535-002-1123-2, indexed in Pubmed: 12898364.
34. Picard D, Infante-Rivard C, Villeneuve JP, et al. Extrahepatic uptake of technetium-99m-phytate: a prognostic index in patients with cirrhosis. J Nucl Med. 1990; 31(4): 436–440, indexed in Pubmed: 2324819.