International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.139.146

Research Article
Effect of Thiamine Pyrophosphate upon Possible Metamizole-Induced Liver Injury in Rats

[1]Zehra Bedir, [2]Kezban Tuna Ozkaloglu Erdem, [1]Abdullah Can, [3]Betul Cicek, [4]Mine Gulaboglu, [5]Zeynep Suleyman, [3]Cebrail Gursul, [6]Behzad Mokhtare, [7]Fatih Ozcicek and [5]Halis Suleyman

[1]Department of Anesthesiology and Reanimation, University of Health Sciences,
Erzurum Regional Education and Research Hospital, Erzurum, Turkey
[2]Department of Anesthesiology and Reanimation, Antalya Provincial Health Directorate,
Antalya Training and Research Hospital, Antalya, Turkey
[3]Department of Physiology, Faculty of Medicine, Erzincan Binali Yildirim University, 24100 Erzincan, Turkey
[4]Department of Biochemistry, Faculty of Pharmacy, Ataturk University, Erzurum, 25030 Yakutiye, Turkey
[5]Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, 24100 Erzincan, Turkey
[6]Department of Pathology, Faculty of Veterinary, Ataturk University, Erzurum, 25030 Yakutiye, Turkey
[7]Department of Internal Medicine, Faculty of Medicine, Erzincan Binali Yildirim University, 24100 Erzincan, Turkey

Abstract

Background and Objective: Metamizole is a non-selective cyclooxygenase (COX) inhibitor NSAID with a strong analgesic and spasmolytic effects. It is the most common analgesic in the world. Thiamine pyrophosphate (TPP) protects the liver tissue against oxidative damage. The aim of our study was to analyze the effect of TPP against possible liver injury and dysfunction of metamizole in rats biochemically and histopathologically. **Materials and Methods:** The animals were grouped as healthy (HG), 500 mg kg^{-1} metamizole (MT-500), 1000 mg kg^{-1} metamizole (MT-1000), 25 mg kg^{-1} TPP+500 mg kg^{-1} metamizole (TMT-500), 25 mg kg^{-1} TPP+1000 mg kg^{-1} metamizole (TMT-1000) groups. The TMT-500 and TMT-1000 groups of animals were injected intraperitoneally (ip) at the dose of 25 mg kg^{-1} of TPP. Distilled water as the solvent was injected ip to the HG, MT-500 and MT-100 groups. One hour after injecting TPP and distilled water, metamizole 500 and 1000 mg kg^{-1} doses were administered orally to the TMT-500 and TMT-1000 groups. The TPP was administered once and metamizole twice a day for 14 days. The animals were euthanized with high-dose anesthesia. The liver tissues excised from the animals were analyzed biochemically and histopathologically. **Results:** In both doses metamizole significantly increased MDA, ALT and AST levels and decreased tGSH, SOD and CAT levels compared to the healthy group. TPP significantly prevented the decrease of tGSH, SOD and CAT levels and the increase of MDA, ALT and AST levels with metamizole. There was no statistical difference in all of the levels between the TMT-500 and TMT-1000 groups. The histopathological findings indicated TPP significantly suppressed the damage induced by metamizole. **Conclusion:** The metamizole was possible to cause moderate damage to liver tissue. Therefore, it was considered that the use of TTP to reduce liver toxicity could be clinically beneficial.

Key words: Analgesic, liver injury, metamizole, rat, thiamine pyrophosphate

Citation: Bedir, Z., K.T.O. Erdem, A. Can, B. Cicek and M. Gulaboglu *et al*., 2023. Effect of thiamine pyrophosphate upon possible metamizole-induced liver injury in rats. Int. J. Pharmacol., 19: 139-146.

Corresponding Author: Halis Suleyman, Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, 24100 Erzincan, Turkey
Tel:+90 4462261818 Fax:+90 446 2261819

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Metamizole is a non-selective cyclooxygenase (COX) inhibitor NSAID with strong analgesic and spasmolytic effect[1]. Metamizole is also known as a pyrazolone-derivative, non-narcotic analgesic and antipyretic agent[2]. Metamizole is the most common analgesic in the European Union, Latin America and many areas of the world. Metamizole has widely been used for post-operative pain in patients, prolonged colic pain, cancer pain and migraineheadache[3]. In previous studies, metamizole has been reported to be more potent than paracetamol in the improvement of pain, tissue inflammation and oxidative stress induced by surgical trauma[4]. As known, metamizole is metabolized to 4-methylaminoantipyrine hydrolyzed in the gastrointestinal tract and to 4-aminoantipyrine through N-demethylation in the liver[5]. The mechanism of action for metamizole has not been fully known. Some information has indicated that the analgesic effect of metamizole is related to the central nervous system. In some studies, metamizole has been argued to be induced by the reduction of prostaglandin synthesis due to the inhibition of different COX isomers[6]. Metamizole has a relatively positive safety profile in terms of morbidity and mortality when compared to other NSAIDs[1]. Although it is emphasized to be safer when compared to other analgesics especially for the treatment of short-term pain, information about its safety in medium and long-term use is limited[3]. Previous studies have revealed that metamizole is associated with a major risk of liver injury[7]. In addition, it has been proven in previous studies that diclofenac, another NSAID drug other than metamizole, has a toxic effect on the liver[8]. However, there is no sufficient evidence to determine whether metamizole increases liver risk or not[2]. Therefore, further researches are needed to be carried out in order to analyze the potential risks associated with metamizole.

Thiamine pyrophosphate (TPP) investigated in terms of its protective effect against possible liver injury in our study is the active metabolite of Thiamine (TA)[9]. The TPP is also the co-factor of enzymes that play a role in maintaining the cell redox synthesizing NADPH and glutathione[10]. It has been reported in previous experimental studies that TPP protects the liver tissue against oxidative damage inhibiting the reduction of GSH stores in liver[11]. As known, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities increase in liver injury. Furthermore, it has also been known that thiamine pyrophosphate significantly prevents ALT and AST increase induced by oxidative liver injury[12]. All this information indicates that TPP may be beneficial against possible liver damage caused by metamizole. There have been no studies serving the protective effect of TPP against metamizole-induced liver injury in the literature. The aim of our study was to analyze the effect of TPP against possible liver injury and dysfunction of metamizole in rats biochemically and histopathologically.

MATERIALS AND METHODS

Study area: This study was performed at Erzincan Binali Yildirim University, Faculty of Medicine, Erzincan, Turkey from August, 2022 to November, 2022.

Animals: In the experiment, 30 albino Wistar type male rats weighing between 280-290 g were procured from University of Erzincan Binali Yildirim, Experimental Animals Application and Research Center. Before the experiment, the animals were fed with animal feed under appropriate conditions in 12 hrs of light and 12 hrs of darkness at normal room temp (22°C). The procedures were accepted by the Local Animal Experimentation Ethics Committee (Date: 18-08-2022, Number: E-85748827-050.01.04-192459).

Chemicals: In this study, thiopental sodium was procured from IE Ulagay (Turkey), Metamizole from Sanofi Aventis (Turkey) and Thiamine pyrophosphate from Biofarma (Russia).

Experimental groups: The rats were grouped as healthy (HG), 500 mg kg^{-1} metamizole (MT-500), 1000 mg kg^{-1} metamizole (MT-1000), 25 mg kg^{-1} TPP+500 mg kg^{-1} metamizole (TMT-500) and 25 mg kg^{-1} TPP+1000 mg kg^{-1} metamizole (TMT-1000) groups.

Experimental procedure: The TMT-500 (N = 6) and TMT-1000 (N = 6) groups of animals were injected intraperitoneally (ip) at 25 mg kg^{-1} of TPP. Distilled water as the solvent was injected ip to the animals in HG (N = 6), MT-500 (N = 6) and MT-100 (N = 6) groups. One hour after injecting TPP and distilled water, metamizole 500 and 1000 mg kg^{-1} doses were administered orally to the TMT-500 and TMT-1000 groups, respectively, by gavage. The TPP was administered once and metamizole twice a day for 14 days. Afterwards, the blood were taken from the tail veins of all animals for ALT and AST measurements. Subsequently, the rats were euthanized with high-dosage anesthesia (50 mg kg^{-1} thiopental sodium). The liver tissues excised from the animals were analyzed biochemically and histopathologically. Biochemical and histopathological findings obtained from all groups were compared and analyzed between the groups.

Biochemical analyses

Sample preparation: The tissue samples were placed in petri dishes after washing with physiological saline. Then, the tissues were ground into powder in the presence of liquid nitrogen. The tissue samples were homogenized to define superoxide dismutase (SOD) activities, reduced glutathione (GSH), TBARS and protein levels. The supernatants were used for SOD, catalase (CAT), GSH, MDA and protein analyses.

MDA, GSH, SOD, CAT and protein determination: Determination of MDA, GSH and SOD in liver tissues was measured with commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits for experimental animals and each analysis was performed according to the kit instructions (item No. 706002, 703002 and 10009055, Cayman Chemical Company, respectively). The CAT was determined according to the method suggested by Goth[13]. Protein determination was based on the Bradford method.

ALT vs AST analysis in tissue: First, venous blood samples were taken for evaluation into tubes without anticoagulant. After coagulation, the serum was isolated by centrifugation and stored at 80°C until analysis. Using a Cobas 8000 autoanalyzer (Roche Diagnostics GmBH, Mannheim, Germany) with commercially available kits (Roche Diagnostics), serum Alanine Aminotransaminase (ALT) and Aspartate Aminotransferase (AST) activities were evaluated spectrophotometrically for the liver function tests.

Histopathologic analysis: Necropsy for the rats was performed and the liver tissues were determined in 10% neutral formalin solution. After routine alcohol-xylol treatments, the tissues were placed in paraffin blocks. Four micro sections taken on the slides were stained with hematoxylin-eosin and necrosis, mononuclear cell infiltrations and congestion were evaluated. The histopathological damage severity in each tissue was graded between 0-3 (0: Normal, 1: Mild injury, 2: Moderate injury and 3: Severe injury).

Statistical analyses: The results obtained from the experiments were meaned as "Mean Value±Standard Deviation" (x±SD). Shapiro-Wilk tests were used for determining to test whether the groups were distributed normally or not and the homogeneity of the variances was determined by the Levene test. The significance level of the difference between the groups was defined using One-way ANOVA Test. Fisher's *post-hoc* Tukey's HSD Test was performed subsequently. All statistical processes were carried out in "SPSS for Windows 25.0" statistical software and the level of $p<0.05$ was considered significant. For the Figures "GraphPad Prism 8 Software" was used. The difference between the groups was determined with Kruskal Wallis test as one of the nonparametric tests and Mann Whitney U test was performed to define the group that created the difference.

RESULTS

Biochemical analysis results

MDA and tGSH analysis in the liver tissue: Figure 1 shows that, MDA level in liver tissues of the rats treated with metamizole at the doses of 500 and 1000 mg kg^{-1} were determined to be significantly increased when compared to the healthy group ($p<0.001$). It was specified that MDA levels treated with TPP were significantly lower than the groups with metamizole ($p<0.001$). Whereas, there was no statistical difference in MDA level between TMT-500 and TMT-1000 groups ($p>0.05$), MDA level was calculated almost the same between TMT-500 and TMT-1000 groups and healthy group ($p>0.05$) (Table 1).

Moreover, tGSH level in liver tissues of the rats treated with metamizole was found to be lower when compared to the healthy group ($p<0.001$). The TPP treatment significantly prevented the decrease of tGSH level with metamizole ($p<0.001$). There was no statistical difference in tGSH level between TMT-500 and TMT-1000 groups ($p>0.05$). The tGSH level was measured almost the same between TMT-500 and TMT-1000 groups and the healthy group ($p>0.05$).

SOD and CAT analysis in the liver tissue: The SOD and CAT levels in liver tissues of the animals treated with metamizole were determined to be decreased significantly when compared to the healthy group ($p<0.001$). Subsequent to TPP injection, SOD and CAT levels were found to be significantly higher than metamizole-treated groups ($p<0.001$). No statistics difference was found in SOD and CAT levels between TPP 500 and 1000 mg kg^{-1} doses ($p>0.05$). The SOD and CAT levels between TMT-500 and TMT-1000 groups were almost the same ($p>0.05$) (Fig. 2).

ALT and AST analysis in the serum: According to Fig. 3, ALT and AST levels in the serum increased significantly when metamizole was treated at 500 and 1000 mg kg^{-1} doses when compared to the healthy group ($p<0.001$). The TPP significantly decreased the increase in ALT and AST levels with metamizole ($p<0.001$). No statistical difference was noticed when the ALT and AST levels in TMT-500 and TMT-1000 groups were compared with the healthy group ($p>0.05$).

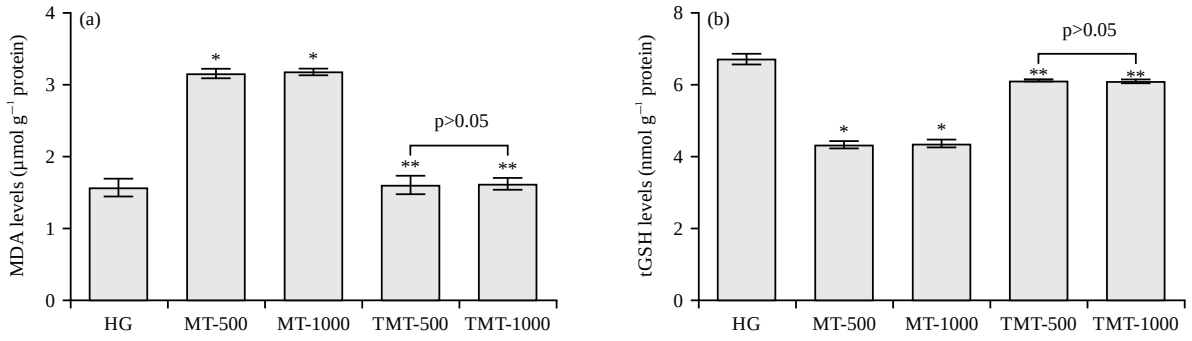

Fig. 1(a-b): MDA and tGSH analysis of the liver tissue in experimental groups. Healthy group (HG), 500 mg kg^{-1} metamizole (MT-500), 1000 mg kg^{-1} metamizole (MT-1000), 25 mg kg^{-1} TPP+500 mg kg^{-1} metamizole (TMT-500) and 25 mg kg^{-1} TPP+1000 mg kg^{-1} metamizole (TMT-1000) groups

*p<0.001 according to HG, MT-500 and MT-1000 groups and **p>0.05 according to HG group, TMT-500 and TMT-1000

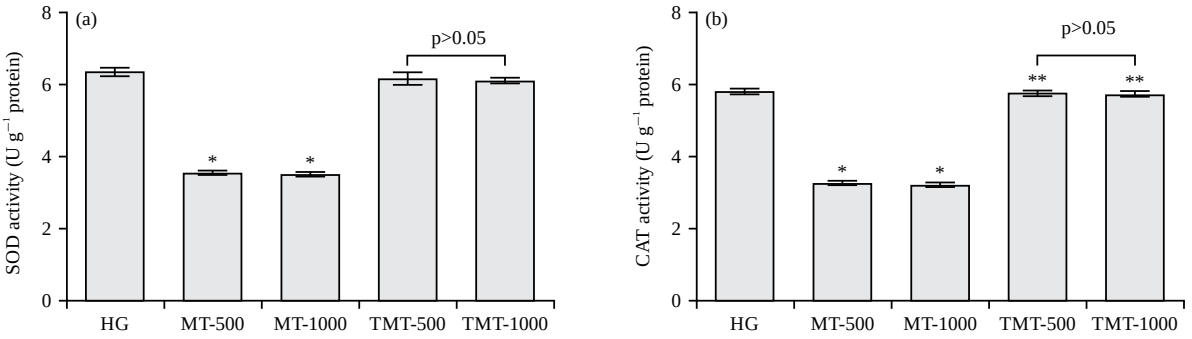

Fig. 2(a-b): SOD and CAT activity of the liver tissue in experimental groups. Healthy group (HG), 500 mg kg^{-1} metamizole (MT-500), 1000 mg kg^{-1} metamizole (MT-1000), 25 mg kg^{-1} TPP+500 mg kg^{-1} metamizole (TMT-500) and 25 mg kg^{-1} TPP+1000 mg kg^{-1} metamizole (TMT-1000) groups

*p<0.001 according to HG, MT-500 and MT-1000 groups and **p>0.05 according to HG group, TMT-500 and TMT-1000

Table 1: Biochemical analysis results in the liver tissue

			Mean±Standard Deviation		
Biochemical parameter	HG	MT-500*	MT-1000*	TMT-500	TMT-1000**
MDA	1.57±0.12	3.16±0.07	3.19±0.04	1.61±0.13	1.62±0.83
tGSH	6.73±0.15	4.34±0.10	3.36±0.11	6.12±0.03	6.10±0.05
SOD	6.37±0.12	3.56±0.06	3.51±0.07	6.18±0.17	6.12±0.08
CAT	5.82±0.08	3.27±0.06	3.22±0.07	5.77±0.08	5.74±0.09
ALT	37.83±3.71	87.50±6.16	83.33±4.55	41.17±4.07	39.83±4.40
AST	36.17±6.11	267.50±6.69	268.50±6.83	47.17±6.74	48.83±9.95

*p<0.001 compared with HG group, **p>0.05 compared with TMT-500. MDA: Malondialdehyde, tGSH: Total glutathione, SOD: Superoxide dismutase, CAT: Catalase, ALT: Alanine aminotransaminase, AST: Aspartate aminotransferase. Healthy group (HG), 500 mg kg^{-1} metamizole (MT-500), 1000 mg kg^{-1} metamizole (MT-1000), 25 mg kg^{-1} TPP+500 mg kg^{-1} metamizole (TMT-500) and 25 mg kg^{-1} TPP+1000 mg kg^{-1} metamizole (TMT-1000) groups (N = 6)

Histopathologic findings: When examined histopathologically, statistically significant differences were found between the groups (Table 2). The liver tissue of the healthy group had a normal appearance (Fig. 4). However, grade-2 necrosis and mononuclear cell infiltrations were observed in hepatocytes (Fig. 5) in liver tissues of the animals in MT-500 and MT-1000 groups treated with metamizole at the doses of 500 and 1000 mg kg^{-1}. The congestion at grade 0-1 interval was observed in the liver tissue of TMT-500 and TMT-1000 groups treated with TPP (Fig. 6).

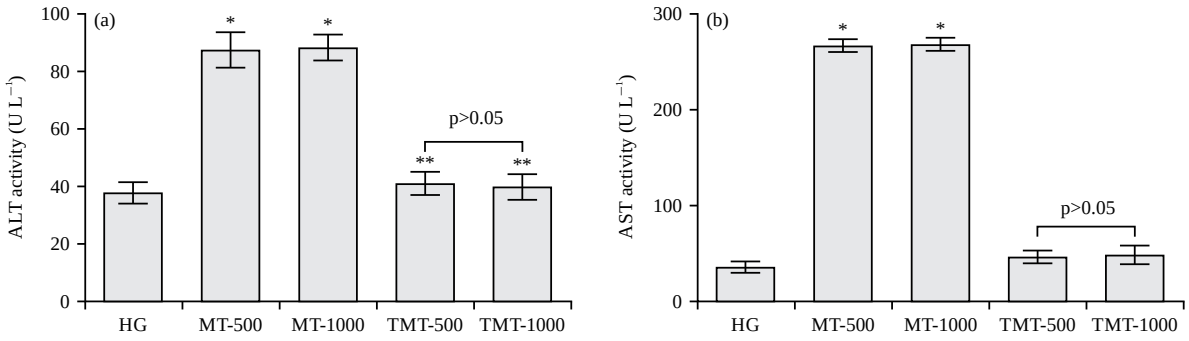

Fig. 3(a-b): ALT and AST levels of the liver tissue in experimental groups. Healthy group (HG), 500 mg kg^{-1} metamizole (MT-500), 1000 mg kg^{-1} metamizole (MT-1000), 25 mg kg^{-1} TPP+500 mg kg^{-1} metamizole (TMT-500) and 25 mg kg^{-1} TPP+1000 mg kg^{-1} metamizole (TMT-1000) groups

*p<0.001 according to HG, MT-500 and MT-1000 groups and **p>0.05 according to HG group, TMT-500 and TMT-1000

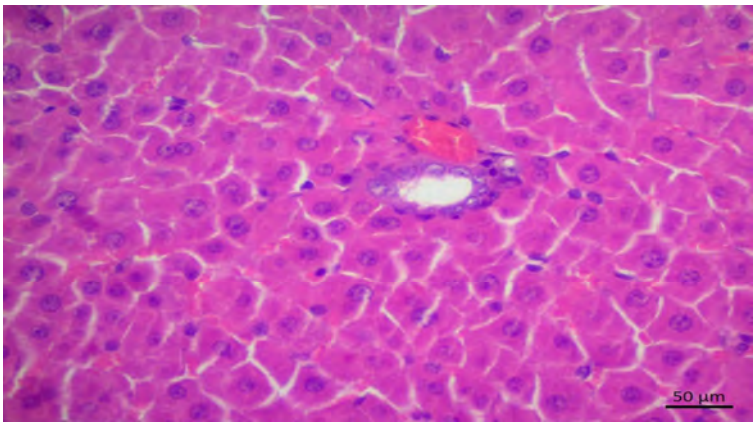

Fig. 4: Normal histological structure of liver tissue of HG group

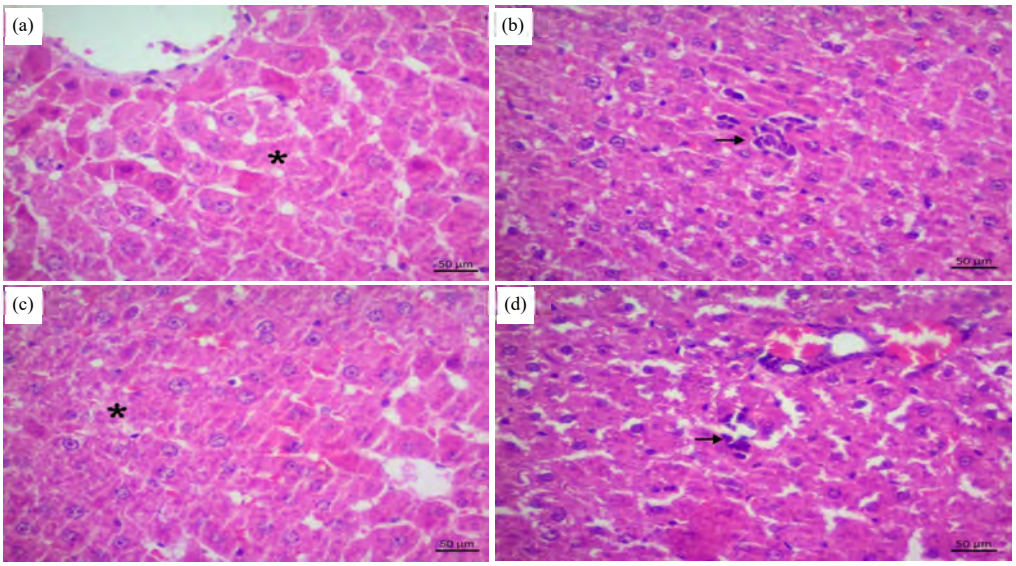

Fig. 5(a-d): Histopathological appearance in liver tissue of the (a-b) MT-500 group and (c-d) MT-1000 group

*Moderate necrosis in hepatocytes (HxE) and Arrow: Moderate mononuclear cell infiltrations evidence (HxE)

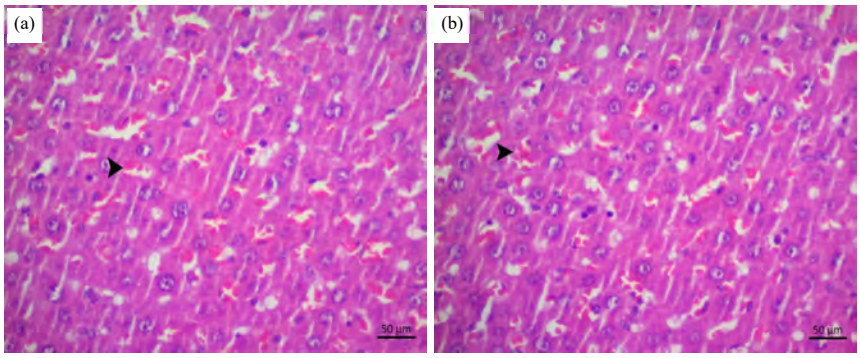

Fig. 6(a-b): (a) Histopathological appearance in liver tissue of the TMT-500 group and (b) TMT-1000 group
Arrowhead: Moderate congestion (HxE)

Table 2: Histopathological examination of liver tissue

Group	Necrosis	Cell Infiltration	Congestion
HG	0 (0-0)	0 (0-0)	0 (0-0)
MT-500	1 (1-2)	2 (1-2)	0 (0-1)
MT-1000	1 (1-2)	2 (1-2)	0 (0-1)
TMT-500	0 (0-0)	0 (0-0)	0 (0-1)
TMT-1000	0 (0-0)	0 (0-0)	0 (0-1)

Histopathological grading, 0: Normal, 1: Mild injury, 2: Moderate injury and 3: Severe injury. Healthy group (HG), 500 mg kg^{-1} metamizole (MT-500), 1000 mg kg^{-1} metamizole (MT-1000), 25 mg kg^{-1} TPP+500 mg kg^{-1} metamizole (TMT-500) and 25 mg kg^{-1} TPP+1000 mg kg^{-1} metamizole (TMT-1000) groups (N = 6). Results are expressed as median (minimum-maximum)

DISCUSSION

In this study, the effect of TPP against possible liver injury and dysfunction of metamizole in rats was analyzed biochemically and histopathologically. The biochemical findings revealed that metamizole at the doses of 500 and 1000 mg kg^{-1} significantly increased the amount of MDA, but significantly decreased the levels of endogenous antioxidants such as tGSH, SOD and CAT in the liver tissue of the rats. The reason to measure the amount of MDA to analyze metamizole-associated liver injury was its being an important indicator for oxidative damage[14]. The MDA as the final product of the LPO reaction impaired cell membrane permeability led to cross-linking of membrane compounds and caused cell damage[15]. It was reported in previous studies that NSAID drugs increased the production of ROS in the liver and caused oxidative damage inducing LPO[16,17]. In the experimental study of Ertekin *et al.*[18], it was expressed that high dose metamizole significantly increased the MDA level in serum samples. In this study, it was determined that metamizole at the doses of 500 and 1000 mg kg^{-1} significantly increased the MDA level in liver tissue of rats in accordance with the literature. High MDA level in the metamizole group indicated that the antioxidant defense system was insufficient to protect the liver tissue against oxidative stress.

Measuring the changes in antioxidant levels was one of the most frequently used methods to elucidate the mechanism of liver toxicity induced by oxygen radicals[19]. Therefore, tGSH level known as the endogenous antioxidant was measured in our study. The GSH as a low molecular weight tripeptide was an important enzyme that acted as a cellular antioxidant and protected the cells from oxidative damage reacting with ROS[20]. Previous studies reported that GSH suppressed LPO in liver microsomes of the experimental animals[21]. Moreover, it was determined that the decrease at GSH amount caused an increase at LPO[22]. As could be understood from our experimental results, tGSH was noticed to be decreased at equal levels in liver tissues of 500 and 1000 mg kg^{-1} metamizole groups. Yapar *et al.*[23] reported that high dose metamizole significantly decreased GSH level in parallel with the increase in oxidant in the liver tissue of mice.

The other enzymatic antioxidants decreased in liver tissues of metamizole group were SOD and CAT in our study. Both SOD and CAT enzymes reduced the oxidative damage breaking down H_2O_2 and reducing superoxide radical as the most effective way to protect the cell from damage. These enzymes acted together to eliminate ROS and the decrease in their activities caused cell structures to be negatively affected[24,25]. As could be noticed from our findings, a significant decrease was determined in SOD and CAT levels in liver tissue of rats as result of both metamizole doses. In the existing literature, there were no studies revealing SOD and CAT decrease in metamizole-induced liver injury. However, there were studies reporting the oxidative damage developed in liver tissue with decreased SOD and CAT enzyme activities[26,27].

The transition of ALT and AST enzymes as the most important biomarkers for the diagnosis of liver diseases to plasma or serum was the most important symptom of liver injury[28]. The increase in serum levels of these enzymes resulted from their mixing with the blood as result of damage to the structural integrity of hepatocytes[29]. It was observed in a

previous study that serum ALT and AST activities increased as result of metamizole-induced liver injury in mice[23]. Furthermore, in a report by Krisai *et al.*[5], hepatotoxicity developed due to the increase in serum ALT and AST activities as result of metamizole treatment. In this study, serum ALT and AST levels were determined to be increased in rats treated with metamizole. We considered that the increase in serum ALT and AST levels was probably induced by oxidative stress.

The TPP that was tested in terms of its effect of metamizole against possible liver toxicity significantly reduced the increase of MDA with metamizole. Yilmaz *et al.*[12] reported that TTP prevented liver injury inhibiting the increase in MDA. Moreover, Delen *et al.*[30] reported that the MDA level increased in rats due to oxidative liver injury induced by propofol decreased significantly as result of TTP treatment. Current findings were coherent with the findings of the studies that associated the hepatoprotective effect of TTP with its antioxidant activity. The TTP also significantly suppressed the decrease in metamizole-induced tGSH, SOD and CAT levels in liver tissue. The experimental results in this study indicated that TTP inhibited the change of oxidant-antioxidant balance in favor of oxidants in liver tissue. It was revealed in the literature that TTP protected the liver tissue from oxidative stress preventing the decrease in antioxidant levels such as tGSH, SOD and CAT[30]. The results of this current study also supported previous studies suggesting that TTP protected the liver tissue against oxidative damage. As result of TTP treatment, ALT and AST levels were found to approach to the normal values in both metamizole groups. Supporting our experimental results, there were studies reporting that TTP prevented the increase in serum ALT and AST levels and alleviated liver injury in the literature[11,12,19].

In this study, the biochemical experiment results were consistent with the histopathological findings. The histopathological findings indicated that necrosis and mononuclear cell infiltrations developed in the hepatocytes of the groups treated at the doses of 500 and 1000 mg kg^{-1}. Supporting these histopathological outputs, the findings such as necrosis, apoptosis, fibrosis, moderate portal and lobular hepatitis were reported in liver tissue of the patients treated with metamizole[31]. Moreover, in this study, treatment with TPP significantly suppressed the damage induced by metamizole. These findings were consistent with the ones in the study carried out by Delen *et al.*[30] reporting that TTP prevented oxidative liver injury induced by propofol.

CONCLUSION

Consequently, it was proved in this study that metamizole caused increase in oxidant and decrease in antioxidants in liver tissue. Moderate level histopathological damage was specified in both metamizole groups with necrosis and moderate mononuclear cell infiltration. Moreover, it was noticed that oxidative liver injury induced by metamizole treatment was reduced by the effect of TTP and antioxidant capacity was protected. The TTP was also indicated to alleviate histopathological damage in liver. Metamizole was possible to cause moderate damage to liver tissue. Therefore, it was considered that the use of TTP to reduce liver toxicity could be clinically beneficial.

SIGNIFICANCE STATEMENT

The purpose of this study was to examine the effect of thiamine pyrophosphate (TPP) against possible liver injury and dysfunction of metamizole in rats. It is known that the NSAID drug diclofenac has a toxic effect on the liver. However, there is no evidence about whether metamizole increases liver damage. This study discovers that TPP protects the liver from metamizole-induced damage by decreasing oxidative variables and increasing antioxidants in rats. It contributes to TPP by reducing the production of ALT and AST. So, a new theory on TPP may be effective in preventing the toxic effects of metamizole on the liver. However, the effect of metamizole on liver tissue at different doses is recommended to be investigated. The dose that produced minimal and maximal toxic effects is necessary to be determined.

ACKNOWLEDGMENT

We would like to thank the Erzincan Binali Yildirim University Experimental Animals Application and Research Center.

REFERENCES

1. van Diepen, A.T.N., P. Simons, J.M. Bos and C. Kramers, 2022. Metamizol: Current status in Dutch practice [Dutch]. Ned. Tijdschr. Geneeskd, Vol. 166.
2. Andrade, S., D.B. Bartels, R. Lange, L. Sandford and J. Gurwitz, 2016. Safety of metamizole: A systematic review of the literature. J. Clin. Pharm. Ther., 41: 459-477.
3. Kötter, T., B.R. da Costa, M. Fässler, E. Blozik and K. Linde *et al.*, 2015. Metamizole-associated adverse events: A systematic review and meta-analysis. PLoS ONE, Vol. 10. 10.1371/journal.pone.0122918.
4. Ince, I., M. Aksoy, A. Ahiskalioglu, M. Comez and A. Dostbil *et al.*, 2015. A comparative investigation of the analgesic effects of metamizole and paracetamol in rats. J. Invest. Surg., 28: 173-180.

5. Krisai, P., D. Rudin, D. Grünig, K. Scherer, W. Pichler, L. Terracciano and S. Krähenbühl, 2019. Acute liver failure in a patient treated with metamizole. Front. Pharmacol., Vol. 10. 10.3389/fphar.2019.00996.
6. Björnsson, E.S., 2020. Liver injury associated with the analgetic drug metamizole. Br. J. Clin. Pharmacol., 86: 1248-1250.
7. Hedenmalm, K., A. Pacurariu, J. Slattery, X. Kurz, G. Candore and R. Flynn, 2021. Is there an increased risk of hepatotoxicity with metamizole? A comparative cohort study in incident users. Drug Saf., 44: 973-985.
8. Ekinci, B., D. Altuner, B. Suleyman, R. Mammadov and S. Bulut *et al*., 2022. Effect of thymoquinone on diclofenac-induced liver injury. Int. J. Pharmcol., 18: 1331-1339.
9. Sambon, M., O. Pavlova, J. Alhama-Riba, P. Wins, A. Brans and L. Bettendorff, 2022. Product inhibition of mammalian thiamine pyrophosphokinase is an important mechanism for maintaining thiamine diphosphate homeostasis. Biochim. Biophys. Acta (BBA) Gen. Subj., Vol. 1866. 10.1016/j.bbagen.2021.130071.
10. Gangolf, M., J. Czerniecki, M. Radermecker, O. Detry and M. Nisolle *et al*., 2010. Thiamine status in humans and content of phosphorylated thiamine derivatives in biopsies and cultured cells. PLoS ONE, Vol. 5. 10.1371/journal.pone.0013616.
11. Demiryilmaz, I., E. Sener, N. Cetin, D. Altuner and B. Suleyman *et al*., 2012. Biochemically and histopathologically comparative review of thiamine's and thiamine pyrophosphate's oxidative stress effects generated with methotrexate in rat liver. Med. Sci. Monit., 18: BR475-BR481.
12. Yilmaz, I., I. Demiryilmaz, M.I. Turan, N. Çetin, M.A. Gul and H. Süleyman, 2015. The effects of thiamine and thiamine pyrophosphate on alcohol-induced hepatic damage biomarkers in rats. Eur. Rev. Med. Pharmacol. Sci., 19: 664-670.
13. Goth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta, 196: 143-151.
14. Kisaoglu, A., B. Borekci, O.E. Yapca, H. Bilen and H. Suleyman, 2013. Tissue damage and oxidant/antioxidant balance. Eurasian J. Med., 45: 47-49.
15. Ayala, A., M.F. Muñoz and S. Argüelles, 2014. Lipid peroxidation: Production, metabolism and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-nonenal. Oxid. Med. Cell. Longevity, Vol. 2014. 10.1155/2014/360438.
16. Galati, G., S. Tafazoli, O. Sabzevari, T.M. Chan and P.J. O'Brien, 2002. Idiosyncratic NSAID drug induced oxidative stress. Chem. Biol. Interact., 142: 25-41.
17. Hermann, M., S. Kapiotis, R. Hofbauer, C. Seelos, I. Held and B. Gmeiner, 1999. Salicylate promotes myeloperoxidase-initiated LDL oxidation: Antagonization by its metabolite gentisic acid. Free Radical Biol. Med., 26: 1253-1260.
18. Ertekin, A., A. Şahin, M. Karaca, H.A Akkan and B. Copper, 2001. The effect of dipyrone overdoses on the levels of lipid peroxidation, glutathione and ceruloplasmin in dogs (Turkish). J. Yuzuncu Yil Univ. Fac. Vet. Med., 12: 105-107.
19. Arauz, J., E. Ramos-Tovar and P. Muriel, 2016. Redox state and methods to evaluate oxidative stress in liver damage: From bench to bedside. Ann. Hepatol., 15: 160-173.
20. Ulrich, K. and U. Jakob, 2019. The role of thiols in antioxidant systems. Free Radical Biol. Med., 140: 14-27.
21. McCay, P.B., D.D. Gibson, K.L. Fong and K.R. Hornbrook, 1976. Effect of glutathione peroxidase activity on lipid peroxidation in biological membranes. Biochim. Biophys. Acta (BBA)-Lipids Lipid Metab., 431: 459-468.
22. Vairetti, M., L.G. di Pasqua, M. Cagna, P. Richelmi, A. Ferrigno and C. Berardo, 2021. Changes in glutathione content in liver diseases: An update. Antioxidants, Vol. 10. 10.3390/antiox10030364.
23. Yapar, K., E. Atakisi, E. Uzlu, O. Atakisi, M. Çitil, M. Uzun and H.M. Erdoğan, 2007. The effect of different doses of metamisole sodium on serum enzyme activities and tissue oxidant levels in liver and kidney in mice [Turkish]. Kafkas Universitesi Veteriner Fakultesi Dergisi, 13: 121-125.
24. Kurutas, E.B., 2015. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr. J., Vol. 15. 10.1186/s12937-016-0186-5.
25. Ighodaro, O.M. and O.A. Akinloye, 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J. Med., 54: 287-293.
26. Taysi, S., K. Gumustekin, B. Demircan, O. Aktas and N. Oztasan *et al*., 2010. *Hippophae rhamnoides* attenuates nicotine-induced oxidative stress in rat liver. Pharm. Biol., 48: 488-493.
27. Wang, R., Z. Yang, J. Zhang, J. Mu, X. Zhou and X. Zhao, 2019. Liver injury induced by carbon tetrachloride in mice is prevented by the antioxidant capacity of anji white tea polyphenols. Antioxidants, Vol. 8. 10.3390/antiox8030064.
28. Lala, V., M. Zubair and D.A. Minter, 2022. Liver Function Tests. In: StatPearls [Internet], Lala, V., M. Zubair and D.A. Minter, StatPearls, USA, pp: 1-27.
29. Seif, H.S.A., 2016. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. Beni-Suef Univ. J. Basic Appl. Sci., 5: 134-146.
30. Delen, L.A., Z.K. Dişli, H.G. Taş, U. Kuyrukluyildiz and G.N. Yazici *et al*., 2022. The effects of thiamine pyrophosphate on propofol-induced oxidative liver injury and effect on dysfunction. Gen. Physiol. Biophys., 41: 63-70.
31. Weber, S., A. Benesic, J. Neumann and A.L. Gerbes, 2021. Liver ınjury associated with metamizole exposure: Features of an underestimated adverse event. Drug Saf., 44: 669-680.