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Effect of morin hydrate on oxidative stress and renal function in methotrexate-induced nephrotoxicity

Seyda Secgin, Tahir Kahraman

Karabük University, Faculty of Medicine, Department of Biochemistry, Karabük, Türkiye

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Abstract

Drugs used for treatment in the clinic also have side effects. As one of these drugs, Methotrexate (MTX) is a folate antagonist antimetabolite that has a wide range of uses in diseases such as cancer and non-cancer autoimmune diseases, inflammatory diseases and abortus. MTX is included in the treatment of diseases such as rheumatoid arthritis, while these patients are known to tend to renal function disorders. Because of this, patients who receive MTX treatment also receive renal function tests periodically and during the treatment process, they are initiated leucovorin, hydration and glucarpidase rescuers, if necessary. Morin hydrate (MH) compound is known to have multi-biological effects such as antioxidant and anti-inflammatory effects. The present study aimed to investigate the effects of a single-effective dose of MTX (20 mg/kg) induced renal toxicity by using the properties of MH (100 mg/kg) compound. In addition to histopathology, renal function tests and oxidative stress/antioxidant levels were evaluated. It was found that while MH improved MTX-induced renal function and structural changes, it also improved MTX-induced renal toxicity significantly. The protective role of MH against MTX-induced renal toxicity was provided due to its free radical scavenging property and by increasing cellular antioxidant defense. We believe that MH is a candidate for protection against renal toxicity of MTX.

Keywords: Renal, morin hydrate, methotrexate, oxidative stress, antioxidant

Introduction

It is a known fact that cancer, which is among the most fought diseases, and which has a very high mortality, does not have a definitive treatment yet [1]. Chemotherapy, surgery, radiotherapy, targeted therapy, and immunotherapy are treatment methods used to prevent the progression of cancer and to improve its prognosis [2]. Another method of treatment which is very common in Chinese medicine, and which is increasingly used around the world is phytotherapy. More comprehensive studies are being carried out for phytotherapy to have a place in medical treatment [3]. Other methods that are being studied on cancer treatment are gene therapies with stem cells and nanoparticles [4]. By general definition, antimetabolites, which are a subclass of antineoplastic agents, affect cell proliferation and drive the cell to apoptosis. After antimetabolites emerged as a successful agent in the treatment of childhood leukaemia and lymphoma in 1950s, antimetabolites also began to be used in the treatment of immune-mediated disorders (rheumatoid arthritis, vasculitis, systemic lupus erythematosus, Chron's disease and abortus) except for malignity and this situation has not changed today [5,6].

MTX, which is a folate analogue, is a highly successful antimetabolite with a wide range of uses. After MTX is taken into the body, it is metabolized in the liver to its more toxic form 7' hydroxy-methotrexate (7'OH-MTX). It is carried to proteins through circulation and then taken into the cell by folate carriers. It is reversibly converted into polyglutamate derivatives (MTX-PGS) inside the cell. This conversion increases the time MTX is in the cell and reveals two different presentations as efficiency and emergence of complications in the cell. Since MTX is a folate antagonist in the cell, its affinity to dihydrofolate reductase enzyme is higher than folate and thus conversion of dihydrofolate to tetrahydrofolate (THF) is inhibited. This inhibition prevents cell proliferation by disrupting the purine and pyrimidine

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Corresponding Author: Tahir Kahraman , Karabük University, Karabük, Faculty of Medicine, Department of Biochemistry, Karabük, Türkiye
Email: tahirkahraman@karabuk.edu.tr

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thymidylate biosynthesis, which is required for ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and adenosine triphosphate (ATP). In addition, since MTX-PGS cannot fulfil its methyl donor function due to the absence of THF in the cell, many syntheses stops and some substances accumulate [6-8]. The events that take place cause release of adenosine into the extracellular fluid, inhibition of polyamine formation and accumulation of homocysteine and 5-amino-imidazole-4-carboxamide ribosyl-5-phosphate (AICAR). These are the mechanisms underlying the anti-inflammatory and immunosuppressive effect of MTX [6,9].

MTX is used for anti-inflammatory and immunosuppressive purposes in low doses, while it is used for antineoplastic purposes in high doses. As a result of studies conducted and clinical use, MTX has been reported to have many complications, including low doses. These complications are mostly seen on mucosal surfaces where cell renewal is high, in the bone marrow, in the liver where it is metabolized and in the kidneys which is the place where it is removed from the body [7-9]. Complications caused by MTX in patients are considered as annoying by both patients and doctors. Therefore, experimental studies are carried out by using natural products to eliminate these side effects [10]. Experimental studies conducted have shown that most of the damage in renal tissue can be fixed with flavonoid compounds that have antioxidant properties. However, some flavonoids or herbal extracts do not show positive effects; on the contrary, they can cause renal toxicity [3]. For this reason, the fact that morin hydrate ($C_{15}H_{10}O_7$ x H_2O, MH) compound, the curative-protective effect of which we examined in the present study, does not show toxicity when used in high doses is in favour of the study [11,12].

Studies have shown that MH has antioxidant and anti-inflammatory effects in renal damage, hepatoxicity, neuroinflammation, aging, testicular mitochondria and sperm cell damage and vascular endothelial damage and also shows apoptotic effects in some cancer cells (leukaemia-colon cancer) [13,14] .

It is a known fact that the number of hydroxyl (OH) groups in flavonoids increase the free radical scavenging effects of that flavonoid. It is stated that MH, with structural nomenclature of 2',3,4',5,7-Pentahydroxyflavone, has a high ability to reduce the oxidants formed with the five OH groups it contains [15]. Morin was originally isolated from members of the Fagaceae, Moraceae, Rosaceae family and can be obtained from the leaves (guava leaf, tea), fruits (white mulberry, old fustic, osage orange, apple peel, onion, almond, fig, sweet chestnut, jack fruit), stems and branches, red wine, seaweed, cereal grains [11].

In the present study, the toxic effects of MTX that occur after accumulation in renal tubules, crystallization and direct exposure to endothelial layer form the basis of our hypothesis. In this context, we planned the present study to examine the effects of MTX in renal tissue and to find out to what extent we can fix the damage that may occur with MH compound.

Material and Methods

Experimental Animals

32 male Wistar albino rats weighing 250-300 g which were used in the study were provided from Bolu Abant Izzet Baysal University Experimental Animal Production and Research Centre. Ethical permission was obtained from Bolu Abant İzzet Baysal University Animal Experiments Local Ethics Committee (Protocol number: 2020/12) for the study in accordance with 28 numbered decisions. During the experiments, the rats were fed with ad libitum pellet feed and water in a standard care room at a temperature of 20-24 C° and 50-60% room humidity in a 12/ hour light-dark environment.

Chemicals and Kits

MTX 50 mg/5 ml was provided from Koçak Farma (İstanbul), MH (2',3,4',5,7-Pentahidroksi-flavon) was provided from Acros Organics (CAS Number: 654055-01-3, Germany), TAS-TOS kits were provided from Rel Assay and all chemicals and reactive were provided from Sigma Aldrich, Merck or other standard suppliers.

Preparation of MH

Morin hydrate was prepared in tris-base HCl buffer (pH=9) and administered as 100 mg/kg through intragastric gavage to each rat in groups 2 and 4.

Experimental Design

Experimental animals were grouped into four groups as eight animals in each group.

Control: Each rat in the group was administered tris-base HCl solution intragastrical for 10 days.

MH: Each rat in the group was administered MH (diluted with tris-base HCl solution) through intragastric gavage at a dose of 100 mg/kg for 10 days [13].

MTX: Each rat in the group was administered 20 mg/kg MTX dissolved in 0.9% NaCl as a single dose intraperitoneally (i.p.) on the first day [16].

MTX+MH: Each rat in the group was administered 20 mg/kg MTX (dissolved in 0.9% NaCl) as a single dose i.p. on the first day. During the experiment, 100 mg/kg MH (in tris-base HCl solvent) was administered as intragastric gavage.

The day after the trial period, experimental groups were anesthetized with 90/10 mg/kg xylazine/ketamine. The serums obtained from blood samples taken by intracardiac puncture by opening the chest cavity of rats were stored at -80 C° until biochemical analysis. After the renal tissues taken from rats were washed with SF, they were frozen in liquid nitrogen and stored at -80 C° until biochemical analysis. Renal tissue was taken in 10% formaldehyde solution for histopathological analyses.

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Tissue Homogenization

Frozen renal tissues were brought to room temperature. The tissues were weighed and homogenized in phosphate buffer at 1/10 (w/v). The homogenates were centrifuged at +4 C°, 5000 rpm for 30 minutes. The resulting supernatants were transferred to Eppendorf tubes.

Biochemical Analyses

Serum renal function tests

Total protein, urea, uric acid and creatinine levels in the serum were analyzed by Siemens brand automatic autoanalyzer. Urea, creatinine, and uric acid results were obtained in mg/dL. Total protein was obtained as g/dL.

Analysis of renal tissue oxidative stress and antioxidant capacity markers

Malondialdehyde (MDA) in kidney tissue was measured by spectrophotometric reading at 532 nm using the thiobarbituric acid reaction method developed by Ohkawa et al.'s [17] for lipid peroxidation assay. Reduced glutathione (GSH) level in kidney tissue was determined by spectrophotometric measurement at 412 nm of the yellow color formed by thiol (-SH) groups with 5.5'-dithiobis-(2-nitrobenzoic acid) developed by Beutler et al.'s [18]. Total antioxidant status (TAS) was calculated by using the Rel Assay Diagnostics Total Antioxidant Status ready-to-use commercial kit developed by Erel [19], which was measured at 660 nm in a spectrophotometer with the reduction of 2,2"-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$) radical by the antioxidants present in the tissue. Total oxidant status (TOS) was determined with the Rel Assay Diagnostics Total Oxidant Status ready-to-use commercial kit developed by Erel [20] by spectrophotometric measurement at 560 nm at 10-minute intervals with the color change reaction realized by the conversion of ferrous (Fe^{+2}) ion to ferric (Fe^{+3}) ion found in the kit reagent of oxidants in the tissue. Oxidative stress index (OSI) was calculated by calculating TOS/TAS ratio.

Histopathological Analyses

Sections taken from paraffin blocks were stained with hematoxylin and eosin (H&E) [21]. and examined for interstitial, tubular, glomerular and vascular lesions under a light microscope (Nikon Eclipse Ci connected to a Hayear® digital camera); They were examined and scored as "no lesion (0), mild lesions (1), moderate lesions (2) and severe lesions (3)".

Statistical Analyses

Minitab (version 17) and SPSS (version 21.0) package programmes were used for all statistical analyses. The conformity of the data to normal distribution was tested by Shapiro Wilk normality test; Mean±standard deviation for normally distributed data and median (min-max) values for non-normally distributed data were included. One-way anova (post hoc; Tukey and Dunnet T3) was used for comparison of normally distributed groups and Kruskal-Wallis H test (post hoc; Pairwise Comparison) was used for comparison of non-normally distributed groups. ($p<0.05$) considered as statistically significant).

Results

Serum Renal Function Test Results

Table 1 shows the biochemical results of urea, creatinine, total protein, and uric acid in serum. No significant correlation was found in urea and creatinine levels, which were examined for renal function ($p>0.05$). With Dunnett T3 test, a significant decrease ($p<0.05$) was found in uric acid levels of MH and MTX+MH groups when compared with MTX group, showing the positive effect of MH on kidney.

Table 1. Serum renal function tests

	Urea mg/dL	Creatinine mg/dL	Total protein g/dL	Uric acid mg/dL
Control	41.13±4.40	0.11±0.07	3.93±1.02	1.26±0.31
MH	42.00±3.12	0.17±0.17	3.71±1.17	0.91±0.42
MTX	56.00±2.51	0.22±0.07	4.98±0.76	2.23±0.89*
MTX+MH	42.63±4.27	0.21±0.10	4.80±0.74	0.91±0.31

Significance in the MH group and MTX-MH group compared to the MTX group *p<0.05 (*p<0.05 MTX vs MH, MTX+MH), Values are mean±standard deviation; n=8
MH: morin hydrate, MTX: methotrexate, MTX-MH: methotrexate- morin hydrate

Biochemical Results of the Renal Tissue

MDA, GSH, TOS, TAS, OSI biochemical results of the renal tissue are shown in Table 2. In the study, while a significant increase ($p<0.05$) was found in MTX group when compared with the other groups in terms of LPO product MDA level, a significant decrease was found in MTX group when compared with the control and MH group in terms of cell antioxidant GSH level ($p<0.05$). In the MTX-MH group treated with MH, GSH level was found to increase

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significantly when compared with the MTX group ($p<0.05$), while TOS and OSI levels were found to decrease significantly ($p<0.05$). TAS level of MH group was found to increase significantly, while OSI level was found to decrease when compared with other groups ($p<0.05$). MTX+MH group TAS level was not significant when compared with control and MTX group ($p>0.05$).

Table 2. Oxidative stress and antioxidant capacity parameters of the renal tissue

	MDA nmol/mg protein	GSH µmol/mg protein	TOS µmol H_2O_2 Equiv/L	TAS mmol Trolox Equiv/L	OSI
Control	1.18±0.27	116.51±33.54	10.51±2.29	1.54±0.20	0.70±0.19
MH	1.10±0.43	122.05±34.36	6.71±2.15****	1.88±0.32****	0.35±0.08****
MTX	2.64±0.9*	65.47±14.95**	12.36±2.54	1.42±0.20	0.87±0.15
MTX+MH	2.02±0.80	76.00±24.83***	8.46±2.00***	1.43±0.24	0.62±0.21***

There is a significant rise in the MDA level in the MTX group compared to other groups (*p<0.05 MTX vs Control, MH, MTX+MH). Antioxidant enzyme GSH levels were lower in the MTX group than in the control and MH groups (**p<0.05 MTX vs Control, MH). MH-treated rats showed a significant increase in GSH level and a significant decrease in TOS and OSI values (***p<0.05 MTX+MH vs MTX). Significant increase (TAS) or significant decrease (TOS, OSI) was reported in the MH group compared to the other groups (****p<0.05 MH vs Control, MTX, MTX+MH). Values are mean±standard deviation; n=8
MH: Morin hydrate, MTX: Methotrexate, MTX-MH: methotrexate- morin hydrate, MDA: malondialdehyde, GSH: glutathione, TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index

Histopathological Results

Table 3 and Figure 1 show the histopathologic results of the renal tissue. Histopathologic analysis revealed normal histologic findings in the renal tissue of the control group, whereas passive congestion was detected in the MH group (Figure; 1A, 1B). In the MTX group, pathologic findings such as congestion, marked hydropic degeneration of tubular epithelium, cystic dilatation, necrosis and hyaline cylinders in tubular lumens, interstitial fibrosis-nephritis, vascular congestion, glomerular inflammation were observed (Figure; 1C, 1D). The highest damage score was found in the MTX group in all findings (Table 3). When the MTX group was compared with the control and MH group, a statistically significant increase was found ($p<0.05$, $p<0.001$). There was a significant decrease in the severity of lesions in the MTX+MH group compared with the MTX group; this decrease was statistically significant especially in the tubular necrosis finding ($p<0.05$).

Table 3. Damage scores of histopathological findings

Groups	Control	MH	MTX	MTX-MH
Histopathologial findings				
Interstitial fibrosis	0.5 (0-1) d*c**	0 (0-1) d*c**	2 (2-2) ab**	1 (1-1) ab*
Interstitial nephritis	0 (0-1) d*c**	0 (0-1) d*c**	2 (2-2) ab**	1 (1-2) ab*
Vascular congestion	1 (0-1) cd**	1 (0-1) cd**	2 (2-2) ab**	2 (2-2) ad**
Tubular degeneration	0.5 (0-1)	0 (0-1) d*c**	1 (1-1) b**	1 (0-1) b*
Hyaline cylinders in the tubul lumen	1 (0-1) d*c**	1 (0-1) c**	2 (1-3) ab**	2 (0-2) a*
Tubular basal membran distortion	0 (0-1)	0.5 (0-1)	0.5 (0-1)	0.5 (0-1)
Glomerular atrophy	0 (0-1)	0 (0-1)	0.5 (0-1)	0 (0-1)
Glomerular inflammation	0 (0-1) c**	0 (0-1) c**	1 (1-1) ab**	0.5 (0-1)
Glomerular hypercellularity	0 (0-1)	0 (0-1)	1 (0-1)	0.5 (0-1)
Glomerular deposits	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Glomerular basal membran distortion	0 (0-1)	0 (0-1)	1 (0-1)	0.5 (0-1)
Tubuler necrosis	0 (0-1) c**	0 (0-1) c**	2 (2-2) ab**d*	1 (0-1) c*

In the evaluation of the data, Kruskal Wallis test was used for multiple comparisons and Pairwise Comparison Post Hoc Test was used for pairwise comparisons. The significance of the differences between the groups is indicated by the letters a, b, c and d for control, MH, MTX and MTX-MH groups, respectively, *p<0.05, **p<0.001 values were considered statistically significant; data are presented as median (min-max) (n=8)
MH: morin hydrate, MTX: methotrexate, MTX-MH: methotrexate- morin hydrate

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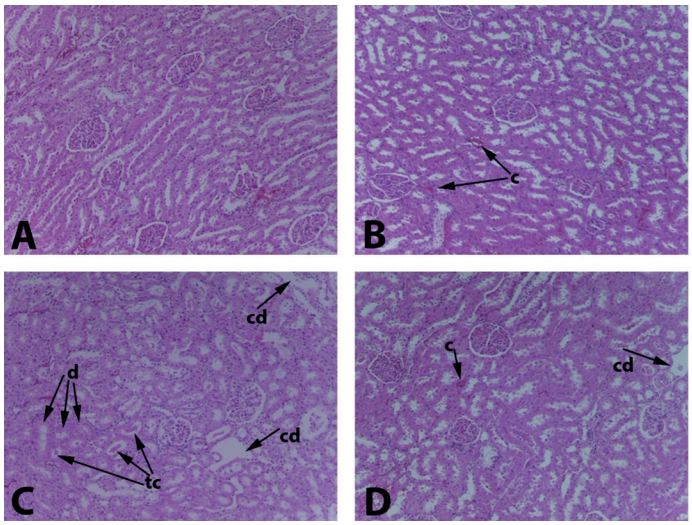

Figure 1. Histopathological sections of renal tissue (Hematoxylin-eosin (H & E) staining method was applied to observe the general histological structure of the kidney. Histopathological findings at X10 magnification (A: Control, B: MH, C: MTX, D: MTX+MH group, c: congestion, cd: cystic dilatation, tc: tubular cast, d: degeneration)

Discussion

The present study showed that MH administration had a protective effect on MTX-induced renal toxicity in rats. It was found in the study that a single dose 20 mg/kg MTX increased uric acid levels among renal function tests and changed the oxidant/antioxidant balance of tissues, in parallel with the results of previously conducted studies [22]. These results were also confirmed with histopathological changes.

MTX is an antimetabolite drug that has a broad range of use in medicine such as neoplasms, autoimmune inflammatory diseases (rheumatoid arthritis, Crohn's disease, systemic lupus erythematosus) and ectopic pregnancy. Due to its cytotoxic feature, MTX causes damage in gastrointestinal system organs, liver, renal and bone marrow, leading to clinical complications. Its toxic effect on the kidneys is directly related to damage in renal tubules, oxidative stress, and free radical formation [23].

In organ failures or damage, significant impairment occurs in blood parameters of the related tissue. The increases in serum urea, creatinine and uric acid levels are renal damage markers [24]. In the study, no significant results were found in MTX groups in terms of renal function markers urea, creatinine and total protein levels measured as a result of MTX induction, when compared with the control group ($p>0.05$). The increase in the results of renal function markers measured in previously conducted studies [25] were not in parallel with the results of the present study. On the other hand, a significant decrease was found in MTX-MH group when compared with the MTX group in terms of uric acid level, which was another parameter ($p<0.05$). The reason why there were no impairments in renal functions in the bloods taken ten days later to evaluate the results of single dose MTX might be due to the half-life of MTX or the fact that it can be compensated as a result of the endogenous defence mechanism of the organism.

In addition, the significant increase in uric acid level included in the study raises a question mark about whether it is a result of renal excretion or a damage in the liver. Therefore, it is thought that analysis of liver tissue or muscle tissue in future studies while examining the serum renal functions of MTX can give more specific information. It was reported in literature that MTX decreased glomerular filtration rate due to exogenous exposure to tissue, precipitation of metabolites in tubules and 90% renal excretion [23]. In addition, in a study examining the pharmacokinetics of MTX (2 mg/kg) with a pre-application of MH (25 mg/kg po), MH was reported to decrease renal clearance and total clearance by 42% and 58%, respectively [26]. At the stage when we established our hypothesis, we thought that impaired renal clearance could be fixed with MH. However, the fact that no significant results were found in the creatinine levels used in renal clearance evaluation prevented us from evaluation in terms of clearance. In the light of this information, it was concluded that a better design of experimental groups and evaluation of blood samples by drawing blood in a period according to the pharmacokinetics of MTX would yield more accurate results in proving in detail that MH may be a compound that contributes positively to renal clearance of MTX.

Kidneys have an important role in maintaining acid-base balance in the body and regulating electrolyte balance by regulating the organic-inorganic substrates in the body. Organic anion transporters (OAT) in the basolateral parts of proximal tubules play an important role in the acid-base system which provides the active elimination of endogenous substances, organic anion compounds and metabolites of these from the body. Precipitation of MTX in renal tissue tubules affects organic anion transporters in the proximal tubules and results in impairment in the buffer system. Hyperuricemia develops with this impairment. In addition, the decrease in the acidic solubility of MTX in acidic medium is a very important information in terms of its pharmacokinetics [7]. In this study, the significant increase in uric acid level of the MTX group ($p<0.05$) supports the information that it results in decreased solubility and precipitation of MTX since it lowers pH.

Improvement in renal function has been reported in the clinic without the need for dialysis by initiating urinary aldolization, leucovorin, hydration and glicarpidase rescuers in toxic plasma dose, delayed renal dysfunction, and HD-MTX treatment where acute renal injury develops (2–12%) [27,28]. MH, the effects of which we examined in the present study, is a compound biochemically close to neutral [29]. According to the results found, it was concluded that the reason why uric acid level in the MTX-MH group was lower than the MTX group was since MH with neutral properties reduced the acidity of the environment.

There are many studies which included MH due to its strong antioxidant properties in preventing and treating renal damage

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[11,30]. Quinone, which is released as a result of MH oxidation, performs a strong antioxidant activity by protecting the unsaturated fatty acids in the cell membrane from oxidation [31]. In addition, the double bond between the C2, C3 atoms of MH and the presence of the OH - group that activates the double bond of C3 have been associated with its antioxidant effect. It is also assumed that the OH group at the 4' position of the B ring is responsible for the radical scavenging effect [11]. In a study investigating the effects of H_2O_2 on the V79-4 cell (Chinese hamster lung fibroblast), cell viability, apoptosis, increase in free radicals and decrease in antioxidant (SOD) enzyme capacity, it was found that MH compound reversed all of these and revealed its free radical scavenging, antioxidant, and anti-apoptotic properties [15].

However, no studies were found on the analysis of antioxidant and oxidative stress parameters TAS, TOS and OSI in studies investigating the effect of MH in MTX-induced renal damage. In a study by Asci et al., [23] investigating the effects of gallic acid (100 mg/kg) in renal tissue induced with MTX (20 mg/kg), TAS, TOS and OSI levels were similar to the results found in this study In the present study, it was found that the results of renal tissue oxidative stress and antioxidant parameters (MDA, GSH, TAS, TOS and OSI) were more significant than the results of serum renal function parameters (urea, uric acid, creatinine, total protein). This shows that the examination of the tissue itself gives more accurate information in the detection of damage in the target tissue because the results are tissue specific. MTX induction causes the formation of free radicals and oxidative stress with effects such as the inability to reduce oxidized glutathione (GSSG) by inhibiting NADPH formation, neutrophil infiltration, and direct chemical toxicity. The increase in MDA, the secondary product of LPO in the disruption of cell membrane integrity, is an important marker of tissue oxidative stress [24]. In an experimental study conducted to reduce hepato-renal damage of MTX with luteolin, it was reported that MDA increase ($p<0.05$) and GSH decrease ($p<0.05$) in the MTX group, and MDA and GSH levels in the group in which luteolin was administered with MTX for therapeutic purposes were reversed [32]. In another study, it was reported that the increase in thiobarbituric acid reagents (MDA, FRAP), the deterioration in morphometric tissue analyses and the xanthine derivative crocin reduced oxidative damage in the experimental animal model in which MTX was applied [16]. In a histopathological study examining renal damage in mitochondrial level, it was found that MTX decreased the level of mitochondrial glutathione [22]. In the present study, the decrease in the levels of GSH, which act as an antioxidant in protecting the redox state of the cell, and the increase in the LPO product MDA value in the MTX group showed that the damage in the renal tissue was caused by oxidative stress. In addition, a decrease in MDA levels and an increase in GSH levels after the application of therapeutic MH were found to be statistically significant ($p<0.05$) in the MTX-MH group compared to the MTX group. It was concluded that MH has an effective therapeutic effect on MTX damage. The results found supported previously conducted studies.

MTX is an agent which is in the indications of many diseases and the elimination of which (90%) takes place in the renal tissue [25]. In the present study, target tissue histopathology analysis was performed to find out the therapeutic role of MH in MTX-induced toxicity in renal tissue. Histopathologic findings such as congestion, marked hydropic degeneration of tubular epithelium, cystic dilatation, tubular necrosis, glomerular inflammation, and hyaline cylinders in tubular and tubular lumens were found in the MTX group compared to the control group. This study showed that renal damage caused by oxidative stress mainly occurs at the histopathological level. Histopathological results in other in vivo studies conducted with MTX are similar to the results of the present study [24,32]. In in vivo studies in which MH is used as a protective agent against damage caused by different toxic agents, histopathological results similar to those in the present study were found. For example, in studies examining cisplatin-induced renal damage, it was reported that hydropic degeneration in the renal, tubular epithelial damage and tubular dilatation findings in the renal were moderately improved and the signs of inflammation were alleviated with the MH compound [33,34]. In the study findings, some statistical results were not given in the table because they were borderline significant, but the improvements in the MH-treated groups were shown by photographs and this information was supported. While the above-mentioned results were found in the MTX group in the present study, a significant decrease in the severity of the lesions was found in the MTX-MH group treated with MH. These results allowed us to obtain data confirming the antioxidant and anti-inflammatory effect of the MH compound.

Conclusion

As a conclusion, tissue damage that occurred as a result of a single effective dose of MTX administration increasing oxidative stress and decreasing antioxidant levels in the renal tissue of rats was confirmed with biochemical and histopathological imaging. The fact that MH compound treated this toxicity proved the antioxidant-oxidant capacity and histopathological analysis results. However, it is thought that it would be useful to carry out such studies and more detailed experimental studies in which MTX and MH are administered together for a long time due to the insignificant results obtained in the renal function test and some parameters in the present study.

Data Availability Statement
I can't share my data for ethical reasons

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

Financial Disclosure
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Ethical Approval
Ethics committee approval was received for the study from Bolu Abant İzzet Baysal University Animal Research Local Ethics Committee (2022/12).

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