The Protective Effect of Rutin Against Methotrexate-Induced Nephrotoxicity in Rats

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ABSTRACT

Objective: This study was purposed to investigate the possible protective effects of rutin on kidney tissue in a methotrexate-induced nephrotoxicity rat model.

Methods: Rats were randomly divided into 4 groups: control group, methotrexate (MTX, 20 mg/kg) group, rutin (RUT, 50 mg/kg/day) group, and methotrexate + rutin (MTX+RUT, 20 mg/kg + 50 mg/kg/day, respectively) group. Biochemical and histopathological analyzes were performed on kidney tissues of rats at the end of the study.

Results: It was determined that kidney tissue damage determined in the MTX group was decreased in the MTX + RUT group; there were no degenerative changes in the tubular epithelium of the MTX + RUT group, and only mild degenerative changes rather than hydropic degeneration seen in MTX group.

Conclusion: This study suggests that rutin can be effective in preventing the toxic effects of methotrexate on the kidney.

Keywords: Methotrexate, nephrotoxicity, rutin

INTRODUCTION

Chemotherapeutic drugs are widely used against various types of cancer, and their clinical use causes side effects, including many organ toxicities. One of these drugs is methotrexate, a folate antagonist. Methotrexate is a commonly prescribed antimetabolite drug used for various cancers and autoimmune diseases. Methotrexate is effective not only in treating cancers but also in various other disorders such as acute leukemia, psoriasis, and rheumatoid arthritis. Over 90% of methotrexate is excreted through the kidneys. Nephrotoxicity is one of the important complications of methotrexate treatment, which is considered to limit the use of methotrexate. Methotrexate causes Bowman capsule cavity enlargement, infiltration of lymphocytes, a decrease in size of glomeruli, an increase in blood cells, degeneration in kidney tubules. Although the etiology of renal dysfunction caused by methotrexate is believed to have a direct toxic effect on kidney tubules, the pathogenesis of methotrexate-induced nephrotoxicity is not yet clear. Neutrophil infiltration and oxidative stress are shown as the cause of cellular damage in methotrexate-induced nephrotoxicity. Flavonoids are natural polyphenolic phytochemicals and are used in the prevention and treatment of many diseases such as cancer, cardiovascular diseases, neurodegenerative diseases, and diabetes. The protective effects of caffeic acid phenethyl ester, carvacrol, gallic acid, propolis, and vitamin C have been shown in kidney damage caused by methotrexate in rats.

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside), also called rutoside and quercetin-3-rutinoside, is a flavonoid found abundantly in plants such as passionflower, buckwheat, tea, and apple. Various pharmacological properties of rutin include antioxidant,
cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, and cardioprotective effects. Some studies have shown that rutin has protective effects on kidneys in diabetic nephropathy, ischemia/reperfusion kidney damage, and drug-induced nephropathy. Rutin has been reported to prevent cisplatin-induced renal inflammation and apoptosis by reducing NFkB, TNF-α, and caspase-3 expression. It has been reported that rutin (50 and 100 mg/kg) protected the kidney with its anti-oxidant, anti-apoptotic, and anti-inflammatory effects against kidney toxicity caused by HgCl₂ in rats.

In the literature, there was no study investigating the possible protective/ameliorative effects of rutin in methotrexate-induced nephrotoxicity in rats. Therefore, the present study aimed to investigate the possible protective/ameliorative effects of rutin against the side effects of methotrexate, which is widely used especially for cancer treatment, on kidney tissue with biochemical analyzes, and histopathological examination in rats.

METHODS

Animals and Experimental Design
Male Wistar Albino rats, weighing approximately 250-300 g, were purchased from the Experimental Research Application and Research Center, Hatay Mustafa Kemal University. The study protocol was approved by the Local Ethical Committee of Experimental Animal Ethics of Hatay Mustafa Kemal University and was performed entirely according to ethical rules (Approval no: 2018/7-2, July 26, 2018). The animals were kept in polypropylene cages under standard temperature (25 ± 2°C), laboratory humidity (45 ± 5%), and 12 hours light/dark cycle conditions during the experiment. The rats were provided ad libitum food and water.

Adult male rats (n = 24) were randomly divided into 4 groups (6 rats for each group).

1. Control Group: The animals were administered orally distilled water for 15 days. Physiological saline (0.09% NaCl) was administered intraperitoneally (i.p.) on the eighth day of the study.
2. Methotrexate (MTX) Group: The animals were administered distilled water orally for 15 days. Methotrexate (Methotrexate, Kocak Pharma, Turkey) was administered in a single dose of 20 mg/kg i.p. on the eighth day of the study.
3. Rutin (RUT) Group: Rutin (rutin hydrate, ABCR, Germany) was administered in a single dose of 50 mg/kg body weight orally, for 15 days. Physiological saline was administered i.p. on the eighth day of the study.
4. Methotrexate + rutin (MTX + RUT) Group: The animals were administered rutin for 15 days. Methotrexate was administered on the eighth day of the study.

Sample Collection and Preparation
At the 16th day of the study, anesthesia was produced by means of a cocktail prepared by using xylazine and ketamine hydrochloride. The rats were sacrificed, kidney tissues were obtained, and used for biochemical and histopathological examinations. One of the kidneys was placed in 10% neutral formalin solution for histopathological investigations while the other kidney of the rat was stored at −80 °C for biochemical analyses.

Preparation of Tissue Homogenates
The kidney tissues were homogenized in phosphate buffer (pH: 7.4) in 1:10 w/v with an ultrasonic homogenizer (Bandelin Electronic UW 2070, Germany) in cooled tubes with ice. Then the homogenates were centrifuged (18 000 rpm, +4°C, 10 min) to obtain tissue homogenate supernatants.

Biochemical Analyzes
Protein measurements were performed using Lowry’s method spectrophotometrically (UV 2100 UV–VIS Recording Spectrophotometer Shimadzu, Japan). Levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the kidney tissue homogenates were detected with a commercial rat ELISA kit (SunRed, China) according to the manufacturer’s instruction. The contents of these parameters were expressed as ng/g tissue. Total glutathione (tGSH) levels were estimated in the kidney tissue homogenates using a commercially available kit (GSH-420™ OxisResearch, USA). The contents of these parameters were expressed as μmol/g protein. Glucose-6-phosphate dehydrogenase (G6PD) activities were determined by Beutler (1971)’s and glutathione peroxidase (GPx) activities were determined by Beutler (1975)’s methods spectrophotometrically. Results were expressed as U/g protein.

Histopathological Examination
The kidney tissues were fixed in a 10% buffered formalin. The fixed tissues were subjected to routine tissue follow-up. They were dehydrated in graded ethanol (50-100%) and were made transparent in xylol. The tissues were embedded in paraffin blocks. Cross-sections of 5 μm thickness were obtained from each block by microtome. After the cross-sections, slides were left in a stove for 1 h, and the preparates were stained by hematoxylin-eosin (HE). The preparates were observed under a light microscope (Olympus CX31, Germany). Later, the structural changes observed in the kidney tissue sections belonging...
and RUT groups were observed to have a normal histological
vations revealed that the kidney tissues of the rats in the control-
findings were summarized in Table 2. Histopathological evalu-
were in normal structure in the other groups. Histopathological
color was lighter than normal in the MTX group, while the kidneys
Macroscopically, the kidneys were slightly swollen, and their
levels as the control group (24.33 ± 1.35 ng/g tissue), MTX (25.30 ± 0.74 ng/g tissue), RUT (23.85 ± 0.86 ng/g tissue), and
MTX + RUT (25.45 ± 1.14 ng/g tissue). The tGSH levels and G6PD
activities did not change within the groups (P > .05). GPx activi-
ties decreased in the RUT group (333.73 ± 36.46 U/g protein, P
< .05 Table 1) compared to the control group (441.78 ± 35.55
U/g protein, Table 1). Rutin administration decreased tissue
G6PD and GPx concentration, but the decrease was not statisti-
cally significant in the MTX + RUT group compared with the MTX
group (Table 1).

Macroscopically, the kidneys were slightly swollen, and their
color was lighter than normal in the MTX group, while the kidneys
were in normal structure in the other groups. Histopathological
findings were summarized in Table 2. Histopathological evalu-
ations revealed that the kidney tissues of the rats in the control
and RUT groups were observed to have a normal histological
appearance (Figure 1A). Small foci mononuclear inflammatory
cell infiltration in the kidney tissue was observed in the MTX
group (Table 2 and Figure 1B). Moreover, there were degener-
ative changes in the tubular epithelium, ranging from paren-
chyma to hydropic, in wide scope in the MTX group (Table 2 and
Figure 1C). In the MTX + RUT group, especially only mild degen-
erative changes were determined rather than hydropic degen-
eration seen in the MTX group (Table 2 and Figure 1D). No lesions
were noted in the glomeruli and vessels in all groups.

RESULTS
The 8-OHdG levels were found to be (Table 1) nearly the same
levels as the control group (24.33 ± 1.35 ng/g tissue) in the MTX
(25.30 ± 0.74 ng/g tissue), RUT (23.85 ± 0.86 ng/g tissue), and
MTX + RUT (25.45 ± 1.14 ng/g tissue). The tGSH levels and G6PD
activities did not change within the groups (P > .05). GPx activi-
tries decreased in the RUT group (333.73 ± 36.46 U/g protein, P
< .05 Table 1) compared to the control group (441.78 ± 35.55
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G6PD and GPx concentration, but the decrease was not statisti-
cally significant in the MTX + RUT group compared with the MTX
group (Table 1).

DISCUSSION
In the present study, the protective and ameliorative effects of
rutin against acute methotrexate toxicity in rat kidneys were
investigated. In the light of the literature review, it has been
reported that administration of methotrexate to rats induces
kidney damage and causes histopathological changes associ-
ated with the damage.1,3,27 Methotrexate can cause changes
ranging from mild tubular degeneration to glomerulosclero-
sis.11,14,28 However, in the present study, no lesions were noted
in the glomeruli and vessels. Mahmoud et al.28 indicated that
methotrexate caused various histopathological changes in
kidney tissues, such as leukocyte infiltration, interstitial hem-
orrhage, and degenerative changes of tubular epithelial cells.
Sakalli et al.11 found renal degenerative changes of methotrex-
ate including tubular distention, interstitial inflammation,
perineal inflammation, glomerular congestion, glomerular
degeneration, and parenchymal bleeding. In keeping with the
previous research, the present study demonstrated that metho-
trexate caused macroscopic and microscopic changes in kid-
ney tissues. The kidneys of the rats administered methotrexate
were lighter in color and slightly swollen, macroscopically.
Macroscopically, degenerations from parenchyma to hydropic
were found in the tubular epithelium in large areas of kidney
tissues. Moreover, mononuclear inflammatory cell infiltration
was observed in small foci.

8-OHdG is a potential biomarker for the early diagnosis of
pathological conditions in the body.29 It has been suggested
that increased risk of kidney disease in individuals with type
1 diabetes is associated with high plasma 8-OHdG levels, and
that 8-OHdG can be used to evaluate the progression of dia-
abetic kidney disease.30 In animal experimental, kidney damage

Table 1. Effects of Methotrexate and Rutin on Some Biochemical Parameters in Rat Kidney

| Groups         | 8-OHdG (ng/g tissue) | tGSH (µmol/g protein) | G6PD (U/g protein) | GPx (U/g protein) |
|----------------|----------------------|-----------------------|--------------------|-------------------|
| Control        | 24.33 ± 1.35         | 72.82 ± 1.14          | 3.32 ± 0.52        | 441.78 ± 35.55<.05|
| MTX            | 25.30 ± 0.74         | 79.15 ± 3.60          | 3.38 ± 0.23        | 410.22 ± 27.17<.05|
| RUT            | 23.85 ± 0.86         | 73.61 ± 2.97          | 2.51 ± 0.43        | 333.73 ± 36.46<.05|
| MTX + RUT      | 25.45 ± 1.14         | 75.61 ± 2.44          | 3.14 ± 0.22        | 375.22 ± 34.15<.05|

Values are expressed as mean ± SE. Different superscripts (a, b, ab) in the same
row indicate significant difference (P < .05) among groups.

MTX, methotrexate; RUT, rutin; 8-OHdG, 8-hydroxy-2’-deoxyguanosine; tGSH, total
glutathione; G6PD, glucose-6-phosphate dehydrogenase; GPx, glutathione
peroxidase.

Table 2. Histopathological Findings and their Scores in Kidney Tissue in Rats

| Groups         | Mononuclear Cell Infiltration | Parenchymal Degeneration | Hydropic Degeneration |
|----------------|-----------------------------|--------------------------|----------------------|
| Control        | −                           | −                        | −                    |
| MTX            | +                           | ++                       | ++                   |
| RUT            | −                           | −                        | −                    |
| MTX + RUT      | −                           | +                        | +                    |

The lesions in the whole area were scored for histopathological changes as −: no
change (<5%), +: mild change (between 5 and 33%) and ++: moderate change
(between 33 and 66%).
is created with various chemicals such as carbon tetrachloride and lead. It has been reported that the mentioned substances may cause an increase in the kidney 8-OHdG level. In cisplatin-induced acute renal failure, it has been revealed that cisplatin causes an increase in 8-OHdG expression in rat distal tubular cells. In the literature searches, no study was found to reveal the effects of methotrexate on kidney 8-OHdG. In the present study, neither methotrexate nor rutin affected kidney 8-OHdG levels in rats. It has been suggested that the significant reduction in GSH levels induced by methotrexate leads to a decrease in the effectiveness of the antioxidant defense system by sensitizing cells to reactive oxygen species. It has been reported that methotrexate-decreased kidney reduced glutathione and tGSH levels. However, in this study, methotrexate did not change kidney tGSH levels. G6PD, the rate-limiting enzyme of the pentose phosphate pathway, is critical for normal cell growth and cell survival as it is the main source of the essential cellular reductant, NADPH. According to literature reviews, changes in G6PD activity have been associated with acute kidney injury and hyperaldosteronism. Increased G6PD activity is reported to be related to all cancers, including kidney cancer. Babiak et al. reported that methotrexate inhibits G6PD and 6-phosphogluconate dehydrogenase activities in HeLa cells. To our knowledge, there was no study on the effects of methotrexate and rutin on kidney G6PD activity in rats. In the present study, it was determined that methotrexate and rutin did not alter kidney G6PD activity. GPx is a cellular enzymatic antioxidant, which reduces lipidic or nonlipidic hydroperoxides as well as hydrogen peroxide while oxidizing GSH. The effects of methotrexate (20 mg/kg, a single dose, i.p.) on rat kidney GPx activity have been reported differently. Abd El-Twab et al. and Savran et al. reported that methotrexate decreased kidney GPx activity whereas Armanag et al. reported unchanged kidney GPx activity in rats. In accordance with the findings of Armanag et al., this study indicated that methotrexate administration did not change kidney GPx activity compared to the control group.

In the studies investigating the effects of rutin on the kidneys, it has been reported that the rutin did not show any negative effects on the glomerulus, tubule cells, and general structure of the kidney. Similar to previous studies, no pathological findings were found in the kidney tissues of rats after rutin administration. It has been reported that rutin did not alter kidney GSH levels. Similar to the researches, it was determined that rutin had no effect on kidney tGSH levels in the present study. Rutin administration did not alter kidney GPx activity at the doses of 50 and 100 mg/kg/day for 7 days; and besides, it increased GPx activity at the dose of 150 mg/kg/day for 14 days. In this study, rutin (50 mg/kg/day for 15 days) caused a decrease in kidney GPx activity. These results may be attributed to the difference in the rutin dose and duration of administration. Rutin administration alone or in combination with methotrexate did not cause any alteration in kidney G6PD activity and 8-OHdG levels. Arjumand et al. (2011) reported that cisplatin induced damages (glomerular and peritubular congestion, increase in inflammatory cells, and necrosis) in the kidney, and administration of rutin plus cisplatin reduced this injury. Similarly, in the present study, mononuclear cell infiltration determined in the MET group was...
not observed in MTX + RUT group, and also hydronic degeneration was decreased.

CONCLUSION
The present study suggests that methotrexate caused damage in the kidney tissue, and rutin may have the potential to protect and ameliorate the kidney from methotrexate-induced nephrotoxicity. Along with methotrexate chemotherapy, rutin can be promising to alleviate the methotrexate-induced nephrotoxicity. Thus, it is recommended to investigate the possible protective and curative effects of rutin on methotrexate-induced nephrotoxicity at different doses and time intervals by further studies.

Ethics Committee Approval: Ethics committee approval was received from the Ethical Committee of Experimental Animal Ethics of Hatay Mustafa Kemal University (Approval no: 2018/7-2, 26/07/2018).

Informed Consent: N/A.

Peer Review: Externally peer-reviewed.

Author Contributions: Concept – F.K., A.K.T.; Design – F.K., A.K.T.; Supervision – F.K.; Analysis and/or Interpretation – A.K.T., T.K., P.P.A., F.K.; Literature Search – F.K., A.K.T.; Writing – F.K., A.K.T.

Acknowledgments: This study was extracted from thesis in Biochemistry Department of Veterinary Medicine Faculty by Ali Kazim Tambağ (master’s degree student).

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

Financial Disclosure: This study was supported by Hatay Mustafa Kemal University Research Fund (grant number: 18.YL.071).

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