Chrysin protects against kidney tissue oxidative damage caused by pemetrexed used in cancer treatment

Dear Editor,

I would like to discuss the potential benefits of chrysin in the context of kidney tissue oxidative damage caused by pemetrexed used in cancer treatment.

Chrysin, a powerful antioxidant compound, is known for its protective effects against oxidative stress. In a recent study, chrysin was found to significantly reduce kidney tissue oxidative damage caused by pemetrexed.

Keywords: Chrysin, Kidney, Oxidative stress

Materials and Methods: A group of rats was divided into two groups: one received pemetrexed, and the other received chrysin in addition to pemetrexed. The kidney tissue oxidative damage was evaluated using biomarkers.

Results: Chrysin significantly reduced the levels of kidney tissue oxidative damage caused by pemetrexed.

Conclusion: Chrysin is a promising candidate for the prevention of kidney tissue oxidative damage caused by pemetrexed.

I believe that further research on the protective effects of chrysin against oxidative stress in kidney tissue would be beneficial.

Yours sincerely,
[Your Name]
literature, including diabetes insipidus besides interstitial nephritis and fibrosis [9].

Chrysin (Chr; 5,7-Dihydroxyflavone / C15H10O4) belongs to natural polyphenols, which are found among others in honey, propolis [10], various medicinal plants and fruits [11, 12]. Chr is a natural antioxidant that prevents cancer formation by reducing the level of free radicals and neutralizing carcinogenic substances [13]. Antioxidants are substances that protect cells immediately or mediate, from the undesirable effects of drugs, carcinogenic agents and toxic radical reactions. Many studies in the literature have shown that Chr has protective effects against damage caused by drugs and toxic agents in various tissues, including the lung, liver, brain and kidney [14-16]. In previous studies on animal models, Chr has been reported to prevent hepatotoxicity from agents such as methotrexate, acetaminophen, doxorubicin and cyclophosphamide through inhibition of oxidative stress and apoptosis [17]. It was shown that Chr improves the damage caused by CCL4 in rat liver and kidney tissues by increasing antioxidant activity and decreasing damage of free radicals [18]. In another study, it was found that Chr, with its antioxidant properties, protected testicular tissue from lead acetate toxicity in rats and brought sperm parameters closer to normal [19].

In various studies, free radicals and increased oxidative stress formation have been reported in kidney damage caused by anticancer agents [20]. Decreases in antioxidant enzyme levels and increases in reactive oxygen species (ROS) production have been reported in experimental PMTX-induced tissue damage model studies [21]. In addition, some studies have reported that high-dose PMTX reduces renal tubular epithelial cell viability, leading to pathological kidney toxicity [22]. Therefore, alternative treatment approaches are being popularized against kidney toxicity caused by PMTX, which is widely used in cancer treatment. The use of natural compounds that are used for medicinal purposes and have antioxidant activity is one of the simplest and most common methods. Like other flavonoids used for this purpose, Chr has various pharmacological effects such as being an antioxidant as well as having anti-inflammatory, antiaging, anticancer and antihypertensive properties. The aim of this study was to investigate the antioxidant effects of Chr against PMTX-induced nephrotoxicity in rats.

Materials and Methods

Animals

The local ethics committee of Inonu University approved the animal experimental protocols and use of animals in this study (2021/12-2). The number of groups and rats in each group (sample size) were determined according to the power analysis based on the values specified. Accordingly, the amount of Type I error was 0.05, the power of the test α (1- β) was 0.8, and the effect size was 0.82 (large). While the number of groups was 5, the minimum sample size required to find a significant difference between the groups was at least 10 animals in each group, the total number was determined as 50 rats [23]. 50 Wistar albino male rats obtained from the Experimental Animals Production and Research Center, Inonu University weighed between 250-300g were randomly separated to 5 groups as: Control, Sham, PMTX, Chr, PMTX+Chr. All rats were individually housed in a temperature-controlled (21±2°C) environment with a 12h/12h light/dark cycle, and they were fed with ad libitum access to a standard laboratory chow diet. Animal care and experimental procedures were performed using methods in accordance with the National Institutes of Health Animal Research Guidelines and ARRIVE guidelines [24].

PMTX and Chr administration

Rats in the PMTX, PMTX+Chr groups were treated with PMTX (ALIMTA, Eli Lilly and Company, Indianapolis, IN, LY231514), multitargeted antifolate, for 4 weeks from the beginning of the study. A weekly dose of freshly prepared 1mg/kg/week PMTX as a solution prepared in 1 ml saline were given 4 dose at to each animal by i.p. [25, 26].

Rats in the Chr and PMTX+Chr groups were treated with Chr (Sigma, CAS No: 480-40-0) for 4 weeks (28 day) from the beginning of the study. A daily dose of freshly prepared 50 mg/kg/ day Chr as a solution prepared in 1 ml vehicle (corn oil) were given at to each animal by oral gavaj [13, 27].

Rats in the Sham groups were 1 ml vehicle (corn oil) were given for 4 weeks (28 day) at to each animal by oral gavaj. PMTX and Chr application started at the same time, 28 days before sacrifice. The study ended with the sacrifice of the rats on the 29th day.

Termination of experiment and collection tissues

At the end of the required period to (4 weeks), the rats were sacrificed under anesthesia (ketamine/xylazine, 80/12 mg/kg), blood samples were collected and kidney tissues split for biochemical analysis. The tissues were stored at -80°C under suitable conditions until the day of the biochemical analysis.

Biochemical analyses

Two hundred milligrams of frozen tissue specimens were homogenized using steel beads (Next Advance BBY24M, Inc. Innovative Lab Products for the Life Sciences, USA) in PBS buffer (1:9, w/v) for approximately 5 min. Malondialdehyde (MDA), which is considered as an indicator of lipid peroxidation, was analyzed by Esterbauer and Cheeseman [28] protocols from the prepared tissue homogenate. The tissue homogenate was centrifuged at 3500g for approximately 45 minutes to remove large debris and supernatant. Antioxidant enzyme activities; Superoxide dismutase (SOD) activity were evaluated as described by Sun et al. [29], protocols from the prepared supernatant. TAS and TOS were measured using TAS/TOS kit sets (Rel Assay Diagnostics kit, Mega Tip, Gaziantep, Turkey) from the prepared kidney tissue supernatant. Biotek HT Snynergy Gen 5 software, immuno plate reader was used for ELISA. Results for TAS measurement tests were calibrated with trolox solution, which is the standard antioxidant and vitamin E analog, calculated in mmol Trolox Equivalent/L units [30, 31]. TAS measurement tests were
calibrated with hydrogen peroxide and the results were expressed as µmol H$_2$O$_2$ equivalent/L [32]. Oxidative stress index (OSI), an indicator parameter of the degree of oxidative stress, was calculated according to TOS/TAS results. Serum BUN and Cr levels were measured using a commercial ELISA kit for rat (SunRed Biotechnology Company, Shanghai, China) according to the manufacturer’s instructions. Results are expressed in U/L for BUN and nmol/ml for Cr.

**Statistical analysis**

The data obtained from the study were made using the biostatapps.inonu.edu.tr/IAY/open access program. The homogeneity of variances in statistical analyses was evaluated with Levene’s test and when it showed normal distribution, multiple comparisons between groups, Tukey HSD was used when variances were homogeneous and Tamhane T2 test was used when they were not. In cases where the assumption of normality was not met, the Kruskal Wallis test was used and the Conover test was used for multiple comparisons. p<0.05 was considered statistically significant.

**Results**

The results of MDA and SOD enzyme activities in kidney tissue are given in Figure 1. The decrease in SOD activity was statistically significant in kidney tissue in the PMTX and PMTX+Chr groups compared to the Control and Sham groups (p<0.05) (Figure 1-A). The decrease of MDA value was statistically significant in the Chr group compared to the Control and Sham groups (p<0.05) (Figure 1-B). The increase of MDA value was statistically significant in the PMTX group compared to Control and Sham groups (p<0.05).

The decrease of TAS level in PMTX group (Figure 2-A) is statistically significant compared to Control, Sham, Chr and PMTX+Chr groups. The increase in TOS levels (Figure 2-B) in the PMTX group was statistically significant compared to Control, Sham, Chr and PMTX+Chr groups (p<0.05). The increase in OSI values (Figure 2-C) in the PMTX group was statistically significant compared to Control, Sham, Chr and PMTX+Chr groups (p<0.05).

The results of BUN and Cr levels in serum is given in Figure 3. The increase in BUN values (Figure 3-A) in the PMTX group was statistically significant compared to Control, Sham, Chr and PMTX+Chr groups (p<0.05). The increase in Cr values (Figure 3-B) in the PMTX group was statistically significant compared to Control, Sham, Chr and PMTX+Chr groups (p<0.05).

**Discussion**

PMTX is a major and widespread cancer drug for malignant pleural mesothelioma treatment. We hypothesized that the systemic exposure would cause damage to kidney tissue when PMTX was i.p. administration. In studies examining tissue damage caused by anticancer drugs, it is suggested that deterioration in the antioxidant/oxidant balance has a considerable role in the damage mechanism [33]. We studied PMTX at once dose weekly for 4 week. Chr, which is known to have antioxidant attributes, was applied daily for 4 weeks against oxidative stress in the kidney tissue due to PMTX use. Our results show that PMTX causes an increase oxidative stress in tissue and BUN and Cr and an increase in serum, which is an indicator of kidney tissue damage. When Chr is used together with PMTX, the damage to the kidney tissue is reduced. This shows that one of the PMTX damage mechanisms may be disruptions in the oxidant/antioxidant balance.

ROS is one of the most important reasons in the mechanism of damage caused by anticancer agents in kidney tissue [34]. The formation of uncontrollable ROS causes...
lipid peroxidation in the cell membrane structure, oxidation of proteins and enzymes [33]. Tissue MDA level is an important and reliable marker of degradation in the oxidation of polyunsaturated fatty acids. Chr has a pronounced protective effect against lipid peroxidation and reduces renal MDA production. Our results are in line with several reports of increased MDA levels in tissues due to PMTX-induced oxidative stress. The potential to scavenge Chr free radicals appears to contribute highly to the inhibition of lipid peroxidation. SOD antioxidant enzyme, which is directly responsible for the detoxification of ROS, is present in high concentration in kidney tissue. PMTX treatment in this study significantly reduced levels of SOD according to current published studies. Considering its antioxidant activity, Chr used in the treatment was similarly able to prevent the decrease in SOD activities in the rat kidney tissue [18]. This effect may be due to an improvement in antioxidant status and scavenging of excess free radicals such as O$_2^-$ and peroxyl radical. Similar results have been obtained with different antioxidants such as melatonin and proanthocyanidin [27] which structurally protect antioxidant enzymes and increase their activity in MTX-induced nephropathy.

Serum Cr concentration is an important marker evaluated in the diagnosis of kidney function. It is more important in clinical assessment than changes in BUN levels in the early stages of kidney disease. On the other hand, BUN starts to rise only after significant damage to kidney parenchymal structures occurs morphologically [34]. It is known that in kidney tissue pathology, ROS changes the filtration surface area, alters the ultrafiltration co-efficient factors that affect glomerular function, and induces mesangial cell contraction. Also, decreased SOD activity causes an increase in O$_2^-$ concentration. Cytotoxic effects of radical oxidant derivatives can lead to glomerular damage. These data suggest that ROS may cause decreased GFR in PMTX-induced kidney injury. Our findings suggest that the protective effect of Chr may be related to its ability to protect, especially by increasing the activity of antioxidant enzymes such as SOD.

TAS and TOS, among the spectrophotometric methods evaluated in our study, are reliable parameters with high sensitivity in determining oxidative stress levels. For biological samples, TAS denotes total antioxidant levels and TOS denotes oxidant levels. The OSI parameter, which is calculated as the TOS/TAS ratio rather than evaluating these parameters alone, is used as the golden indicator of oxidative stress, which is more reliable for the quantitative assessment of redox homeostasis disorders [35]. In this study, increased OSI levels and decreased TAS levels with increased TOS in PMTX-treated rats indicate that PMTX-induced oxidative cell damage is mediated by ROS. In addition, Chr treatment with PMTX increased the TAS level and decreased the OSI level, thereby reducing the damage.

In the literature, it has been reported that the application of chrisin helps the treatment by increasing the antioxidant activity in studies of kidney damage due to toxicity [36]. Our study findings add a new data to the studies in the literature and show that PMTX may be effective in reducing kidney damage due to use. Anticancer agents are the most common treatment method in cancer treatments. In such a treatment process, the use of Chr together with PMTX may reduce peripheral tissue damage.

### Conclusion

Consequently, our study findings and previous studies have shown that PMTX increased oxidative stress in kidney tissue and Chr is a strong antioxidant. However, thanks to its antioxidant activity, it reduced the damage caused damage in lipid and protein structures by increased oxidative stress by PMTX. It may have been effective in reducing the damage in the kidney tissues by acting on apoptotic, autophagic and mitophagic pathways, which we did not evaluate in our study.

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### Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

### Author’s contributions

Conceptualization, K.T.; methodology, K.T. and E.K. software, K.T.; formal analysis, K.T.; data curation K.T., writing, original draft preparation, K.T. and E.K.; All authors have read and agreed to the published version of the manuscript.

### Ethical approval

This study was carried out with approval of Ethical Committee of Experimental Animals of the Faculty of Medicine in Inonu University (2021/12-2). The authors have no ethical conflicts to disclose.

### Declaration of competing interests/Conflict of interest

The authors declare that they have no competing interests.

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