Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

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

Kevser Tanbek[a,*], Evren Kose[b]

[a] *Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Türkiye*
[b] *Inonu University, Faculty of Medicine, Department of Anatomy, Malatya, Türkiye*

ARTICLE INFO

Keywords:
Pemetrexed
Chrysin
Kidney
Oxidative stress
Rat

Received: Oct 25, 2023
Accepted: Nov 22, 2023
Available Online: 27.11.2023

DOI:
10.5455/annalsmedres.2023.10.290

Abstract

Aim: Pemetrexed (PMTX) is a multi-targeted anticancer agent that exerts its antifolate effect by disrupting the folate dependent metabolic processes underlying cell proliferation. Kidney toxicity is a common side effect of anticancer agents. Chrysin (Chr) is a powerful antioxidant compound abundant in plant extracts, honey, and bee propolis. The aim of this study is to investigate the effect of the combined use of chrysin, a natural flavonoid, against the possible harmful effects of PMTX on kidney tissue.

Materials and Methods: 50 Wistar albino male rats were divided; Control, Sham, PMTX, Chr, PMTX+Chr groups. Sham (1ml corn oil/day), Chr (50mg/kg/day) by oral gavage, PMTX (1mg/kg/week) by i.p., PMTX+Chr (PMTX;1mg/kg/week, Chr;50mg/kg/day) were given at the same time every day. At the end of 4 weeks of the study, kidney tissues and blood were collected. Creatinine (Cr) and blood-urea-nitrogen (BUN) analyzed in serum by ELISA. The malondialdehyde (MDA), superoxide dismutase (SOD) activities and total antioxidant status (TAS), total oxidant status (TOS), OSI also were measured in kidney tissue.

Results: Indicators of oxidative stress, MDA was elevated and antioxidant activity was reduced in the PMTX groups compared to Control and Sham groups ($p<0.05$). In the PMTX+Chr group, MDA, BUN, Cr and TOS were decreased, SOD and TAS was increased compared to PMTX group ($p<0.05$).

Conclusion: Chr exhibited ameliorative effects on PMTX induced nephrotoxicity increasing antioxidant activity and reducing oxidative damage.

Introduction

Nephrotoxicity can be caused by acute and long-term exposure to toxic chemicals, and it is also defined as the toxic effect of some drugs used for treatment on the kidneys. In patients with impaired renal function, the nephrotoxic effects of most drugs are more pronounced. In patients receiving cancer treatment, if there is underlying kidney disease, avoiding drugs and interventions with nephrotoxic potential, making necessary dose reductions, ensuring adequate hydration, and co-administration of protective agents against the damage mechanisms that the drug may cause are the most important precautions that can be taken to protect against drug nephrotoxicity [1].

Pemetrexed (PMTX) is a multi-targeted antimetabolite that exerts its anticancer effect by inhibiting various enzymes (dihydrofolate reductase, thymidylate synthase and glycinamide ribonucleotide formyltransferase) that are connected in folate synthesis at the base of cell proliferation [2]. This agent has broad antitumor activity in a wide variety of solid tumors, including big cell lung, breast, pancreatic, stomach, colorectal, and bladder. PMTX is used as primary therapy in combination with cisplatin aganist especially in metastatic big cell lung cancer [3] and treatment of malignant mesothelioma [4].

PMTX toxicity has been reviewed in many studies. PMTX may cause toxicity development by affecting liver cells [5]. It can inhibit bone marrow, which manifests itself with anemia. Myelosuppression is usually dose-limiting toxicity [6]. Other side effects seen; dehydration, asthenia, neutropenia, sepsis, nausea, skin urticaria, diarrhea and fever [6]. Many cases have been published documenting acute kidney injury and decreased kidney function with PMTX [7]. Acording a clinical study, phase III studies, 2.4% of patients developed all degrees of renal failure and 0.6% developed conditions requiring dialysis [8]. Several cases of PMTX induced tubular injury have been reported in the

*Corresponding author:
Email address: kevser.tanbek@inonu.edu.tr (Kevser Tanbek)

literature, including diabetes insipidus besides interstitial nephritis and fibrosis [9].

Chrysin (Chr; 5,7-Dihydroxyflavone / $C_{15}H_{10}O_4$) 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 mediately, 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 CCl_4 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 on experimental animal research 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 seperated 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 tisues 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 steal beads (Next Advance BBY24M, Inc. Innovative Lab Products for the Life Sciences, USA) in PBS buffer (1:9, w/v) for approximately 5 min. Malondoaldehyde (MDA), which is considered as an indicator of lipid peroxidation, was analyzed by Esterbauer and Cheeseman [28] protocols from the prepared tissue homegenate. 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, immino 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]. TOS measurement tests were

calibrated with hydrogen peroxide and the results were expressed as µmol H_2O_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 analyzes 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 H 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 ($p<0.05$).

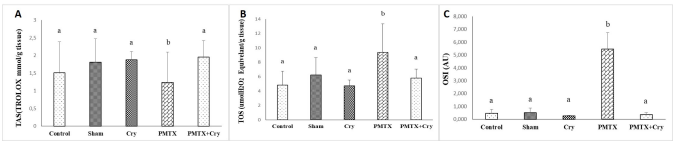

Figure 1. Descriptive statistical criteria for the levels of MDA (A) and the activities of SOD (B) parameters in kidney tissues. Data are given as mean±SD and comparison between groups was made with Kruskal Wallis test. a, b, c the difference between the group with different superscript letters on the same line is statistically significant($p<0.05$) (n=10).

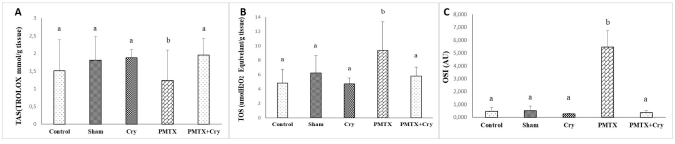

Figure 2. Descriptive statistical criteria for the activities of TAS (A), TOS (B), OSI (C) parameters in kidney tissues. Data are given as mean±SD and comparison between groups was made with Kruskal Wallis test. a, b, c the difference between the group with different superscript letters on the same line is statistically significant($p<0.05$) (n=10).

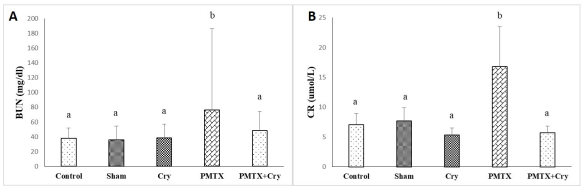

Figure 3. Descriptive statistical criteria for the levels of BUN (A), Cr (B) parameters in kidney tissues. Data are given as mean±SD and comparison between groups was made with Kruskal Wallis test. a, b, c the difference between the group with different superscript letters on the same line is statistically significant($p<0.05$) (n=10).

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 levsels 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 had 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 tisue [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 increced oxidatif 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.

Acknowledgements

Not applicable.

Funding

This work was supported by the The Research Support Unit of Inonu University (Project Number: TYL-2021-2632).

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.

References

1. Ekici K, Temelli O, Parlakpinar H, Samdanci E, Polat A, Beytur A, et al. Beneficial effects of aminoguanidine on radiotherapy-induced kidney and testis injury. Andrologia. 2016;48(6):683-92. doi: 10.1111/and.12500.
2. Shih C, Habeck LL, Mendelsohn LG, Chen VJ, Schultz RM. Multiple folate enzyme inhibition: mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). Adv Enzyme Regul. 1998;38:135-52. doi: 10.1016/s0065-2571(97)00017-4.
3. Shi YK, Wang L, Han BH, Li W, Yu P, Liu YP, et al. First-line icotinib versus cisplatin/pemetrexed plus pemetrexed maintenance therapy for patients with advanced EGFR mutation-positive lung adenocarcinoma (CONVINCE): a phase 3, open-label, randomized study. Ann Oncol. 2017;28(10):2443-50. doi: 10.1093/annonc/mdx359.
4. Rossi G, Alama A, Genova C, Rijavec E, Tagliamento M, Biello F, et al. The evolving role of pemetrexed disodium for the treatment of non-small cell lung cancer. Expert opinion on pharmacotherapy. 2018;19(17):1969-76. doi: 10.1080/14656566.2018.1536746.

5. Temel Y, Kucukler S, Yildirim S, Caglayan C, Kandemir FM. Protective effect of chrysin on cyclophosphamide-induced hepatotoxicity and nephrotoxicity via the inhibition of oxidative stress, inflammation, and apoptosis. Naunyn Schmiedebergs Arch Pharmacol. 2020;393(3):325-37. doi: 10.1007/s00210-019-01741-z.
6. de Rouw N, Piet B, Derijks HJ, van den Heuvel MM, Ter Heine R. Mechanisms, Management and Prevention of Pemetrexed-Related Toxicity. Drug Saf. 2021;44(12):1271-81. doi: 10.1007/s40264-021-01135-2.
7. Dumoulin DW, Visser S, Cornelissen R, van Gelder T, Vansteenkiste J, von der Thusen J, et al. Renal Toxicity From Pemetrexed and Pembrolizumab in the Era of Combination Therapy in Patients With Metastatic Nonsquamous Cell NSCLC. J Thorac Oncol. 2020;15(9):1472-83. doi: 10.1016/j.jtho.2020.04.021.
8. de Rouw N, Boosman RJ, van de Bruinhorst H, Biesma B, van den Heuvel MM, Burger DM, et al. Cumulative pemetrexed dose increases the risk of nephrotoxicity. Lung Cancer. 2020;146:30-5. doi: 10.1016/j.lungcan.2020.05.022.
9. Awad MM, Gadgeel SM, Borghaei H, Patnaik A, Yang JC, Powell SF, et al. Long-Term Overall Survival From KEYNOTE-021 Cohort G: Pemetrexed and Carboplatin With or Without Pembrolizumab as First-Line Therapy for Advanced Nonsquamous NSCLC. J Thorac Oncol. 2021;16(1):162-8. doi: 10.1016/j.jtho.2020.09.015.
10. Woźniak M, Mrówczyńska L, Kwaśniewska-Sip P, Waśkiewicz A, Nowak P, Ratajczak IJM. Effect of the solvent on propolis phenolic profile and its antifungal, antioxidant, and in vitro cytoprotective activity in human erythrocytes under oxidative stress. 2020;25(18):4266.
11. Lopes AP, Galuch MB, Petenuci ME, Oliveira JH, Canesin EA, Schneider VVA, et al. Quantification of phenolic compounds in ripe and unripe bitter melons (Momordica charantia) and evaluation of the distribution of phenolic compounds in different parts of the fruit by UPLC–MS/MS. 2020;74(8):2613-25.
12. Sharma P, Kumari A, Gulati A, Krishnamurthy S, Hemalatha SJNn. Chrysin isolated from Pyrus pashia fruit ameliorates convulsions in experimental animals. 2019;22(8):569-77.
13. Rashno M, Sarkaki A, Farbood Y, Rashno M, Khorsandi L, Naseri MKG, et al. Therapeutic effects of chrysin in a rat model of traumatic brain injury: A behavioral, biochemical, and histological study. Life Sci. 2019;228:285-94. doi: 10.1016/j.lfs.2019.05.007.
14. Pingili RB, Pawar AK, Challa SR, Kodali T, Koppula S, Toleti V. A comprehensive review on hepatoprotective and nephroprotective activities of chrysin against various drugs and toxic agents. Chem Biol Interact. 2019;308:51-60. doi: 10.1016/j.cbi.2019.05.010.
15. Rashid S, Ali N, Nafees S, Ahmad ST, Arjumand W, Hasan SK, et al. Alleviation of doxorubicin-induced nephrotoxicity and hepatotoxicity by chrysin in Wistar rats. Toxicol Mech Methods. 2013;23(5):337-45. doi: 10.3109/15376516.2012.759306.
16. Mishra A, Mishra PS, Bandopadhyay R, Khurana N, Angelopoulou E, Paudel YN, et al. Neuroprotective Potential of Chrysin: Mechanistic Insights and Therapeutic Potential for Neurological Disorders. Molecules. 2021;26(21). doi: 10.3390/molecules26216456.
17. Zhou YJ, Xu N, Zhang XC, Zhu YY, Liu SW, Chang YN. Chrysin Improves Glucose and Lipid Metabolism Disorders by Regulating the AMPK/PI3K/AKT Signaling Pathway in Insulin-Resistant HepG2 Cells and HFD/STZ-Induced C57BL/6J Mice. J Agric Food Chem. 2021;69(20):5618-27. doi: 10.1021/acs.jafc.1c01109.
18. Baykalir BG, Arslan AS, Mutlu SI, Parlak Ak T, Seven I, Seven PT, et al. The protective effect of chrysin against carbon tetrachloride-induced kidney and liver tissue damage in rats. Int J Vitam Nutr Res. 2021;91(5-6):427-38. doi: 10.1024/0300-9831/a000653.
19. Zhandi M, Ansari M, Roknabadi P, Zare Shahneh A, Sharafi M. Orally administered Chrysin improves post-thawed sperm quality and fertility of rooster. Reprod Domest Anim. 2017;52(6):1004-10. doi: 10.1111/rda.13014.
20. de Azevedo Queiroz IO, Machado T, Alves CC, Vasques AMV, Cury MTS, Vasconcelos BC, et al. Tracing the toxic ions of an endodontic tricalcium silicate-based sealer in local tissues and body organs. J Trace Elem Med Biol. 2021;68:126856. doi: 10.1016/j.jtemb.2021.126856.
21. Ji S, Ma Y, Xing X, Ge B, Li Y, Xu X, et al. Suppression of CD13 Enhances the Cytotoxic Effect of Chemotherapeutic Drugs in Hepatocellular Carcinoma Cells. Front Pharmacol. 2021;12:660377. doi: 10.3389/fphar.2021.660377.
22. Boosman RJ, Dorlo TPC, de Rouw N, Burgers JA, Dingemans AC, van den Heuvel MM, et al. Toxicity of pemetrexed during renal impairment explained-Implications for safe treatment. Int J Cancer. 2021;149(8):1576-84. doi: 10.1002/ijc.33721.
23. Tanbek K, Ozerol E, Gul M. Effects of Alpha Lipoic Acid Learning Behaviors and Histological Examinationon Brain Tissue on Diabetic rats. Acta Physiologica. 2017;221:110-.
24. Çolak C, PARLAKPINAR HJJoTOMC. Hayvan deneyleri: in vivo denemelerin bildirimi: ARRIVE Kılavuzu-Derleme. 2012;19(2):128-31.
25. Skalska S, Kucera P, Goldenberg Z, Stefek M, Kyselova Z, Jariabka P, et al. Neuropathy in a rat model of mild diabetes induced by multiple low doses of streptozotocin: effects of the antioxidant stobadine in comparison with a high-dose alpha-lipoic acid treatment. Gen Physiol Biophys. 2010;29(1):50-8.
26. Pestieau SR, Stuart OA, Sugarbaker PH. Multi-targeted antifolate (MTA): pharmacokinetics of intraperitoneal administration in a rat model. Eur J Surg Oncol. 2000;26(7):696-700. doi: 10.1053/ejso.2000.0983.
27. Kandemir FM, Kucukler S, Eldutar E, Caglayan C, Gulcin I. Chrysin Protects Rat Kidney from Paracetamol-Induced Oxidative Stress, Inflammation, Apoptosis, and Autophagy: A Multi-Biomarker Approach. Sci Pharm. 2017;85(1). doi: 10.3390/scipharm85010004.
28. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol. 1990;186:407-21. doi: 10.1016/0076-6879(90)86134-h.
29. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem. 1988;34(3):497-500.
30. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical biochemistry. 2004;37(4):277-85. doi: 10.1016/j.clinbiochem.2003.11.015.
31. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clinical biochemistry. 2004;37(2):112-9. doi: 10.1016/j.clinbiochem.2003.10.014.
32. Erel O. A new automated colorimetric method for measuring total oxidant status. Clinical biochemistry. 2005;38(12):1103-11. doi: 10.1016/j.clinbiochem.2005.08.008.
33. Huang G, Zhang Q, Xu C, Chen L, Zhang H. Mechanism of kidney injury induced by cisplatin. Toxicol Res (Camb). 2022;11(3):385-90. doi: 10.1093/toxres/tfac019.
34. Vardi N, Parlakpinar H, Ates B, Cetin A, Otlu A. The protective effects of Prunus armeniaca L (apricot) against methotrexate-induced oxidative damage and apoptosis in rat kidney. J Physiol Biochem. 2013;69(3):371-81. doi: 10.1007/s13105-012-0219-2.
35. Mentese A, Alemdar NT, Livaoglu A, Ayazoglu Demir E, Aliyazicioglu Y, Demir S. Suppression of cisplatin-induced ovarian injury in rats by chrysin: an experimental study. J Obstet Gynaecol. 2022:1-7. doi: 10.1080/01443615.2022.2130201.
36. Samarghandian S, Farkhondeh T, Azimi-Nezhad M. Protective Effects of Chrysin Against Drugs and Toxic Agents. Dose Response. 2017;15(2):1559325817711782. doi: 10.1177/1559325817711782.