Volume 13, Issue 3, Page 140-147, 2024 https://doi.org/10.46810/tdfd.1511831 Research Article

BİNGÖL UNIVERSITY

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Investigation of the Effects of Rutin in Sprague Dawley Rats with Biochemical Parameters in Colistin-Induced Lung Injury

Özge KANDEMİR[1*], Sefa KÜÇÜKLER[2]

[1] Aksaray University, Aksaray Vocational School of Technical Sciences, Food processing Department, Aksaray, Türkiye
[2] Atatürk University, Veterinary Faculty, Veterinary Biochemistry Department, Erzurum, Türkiye
Özge KANDEMİR ORCID No: 0000-0001-8884-4168
Sefa KÜÇÜKLER ORCID No: 0000-0002-8222-5515

**Corresponding author: ozgekandemir@aksaray.edu.tr*

(Received: 07.07.2024, Accepted: 06.09.2024, Online Publication: 26.09.2024)

Keywords
Lung injury, Colistin, Oxidative stress, Rutin

Abstract: Colistin (COL), frequently used for Gram-negative bacteria, may cause pulmonary toxicity in a dose-dependent manner. Flavanoid-type antioxidants have started to be used frequently against toxicity caused by different chemical agents. Rutin (RUT) is one of the flavanoid-type antioxidants. The present study aimed to investigate the effects of RUT in rats with COL-induced lung injury using biochemical parameters. In the experiment, 35 Sprague Dawley rats were divided into five groups (n=7): Control, RUT, COL, COL+RUT50, and COL+RUT100. It was determined that COL increased lung tissue MDA values, decreased SOD, CAT, GPx activities, and GSH values, and triggered oxidative stress. COL administration increased NF-κB, TNF-α, IL-1β, MPO, and COX-2 levels, decreased mTOR levels, increased Beclin-1 levels and accelerated autophagy, increased Caspase-3 activity, and induced apoptosis. It was determined that RUT administration suppressed oxidative stress, inflammation, autophagy, and apoptosis by reversely regulating all these markers and reducing cell damage. The findings showed that the RUT application would be useful in COL-induced lung injury.

Kolistin ile Akciğer Hasarı Oluşturulan Sprague Dawley Ratlarda Rutin'in Etkilerinin Biyokimyasal Parametreler ile Araştırılması

Anahtar Kelimeler
Akciğer hasarı, Kolistin, Oksidatif stres, Rutin

Öz: Gram negatif bakteriler için sıklıkla kullanılan kolistin (COL) doza bağımlı olarak akciğer toksisitesinede neden olabilmektedir. Flavanoid türü antioksidanlar farklı kimyasal ajanların neden olduğu toksikasyonlara karşı günümüzde oldukça sık kullanılmaya başlamıştır. Rutin (RUT) flavanoid türü antioksidanlardan biridir. Sunulan çalışmada COL ile akciğer hasarı geliştirilen ratlarda RUT'in etkilerinin biyokimyasal parametreler ile araştırılması amaçlanmıştır. Deneyde 35 adet Sprague Dawley rat kontrol, RUT, COL, COL+RUT 50 ve COL+RUT100 olmak üzere 5 gruba (n=7) ayrıldı. COL' in akciğer dokusu MDA değerlerini artırıp, SOD, CAT, GPx aktiviteleri ile GSH değerlerini azalttığı ve oksidatif stresi tetiklediği tespit edildi. COL uygulamasının, inflamasyon belirteçlerinden NF-□B, TNF-α, IL-1β, MPO ve COX-2 seviyelerini artırdığı, mTOR düzeylerinin azalıp ve Beclin-1 seviyelerini yükselterek otofajiyi hızlandırdığı, Kaspaz-3 aktivitesini artırarak apoptozisi indüklediği saptandı. RUT uygulamasının tüm bu belirteçleri tersine regüle ederek oksidatif stres, inflamasyon, otofaji ve apoptosizi baskıladığı ve hücredeki hasarı azalttığı belirlendi. Elde edilen bulgular COL kaynaklı akciğer hasarında RUT uygulamasının yararlı olacağını gösterdi.

1. INTRODUCTION

Antimicrobial resistance (AMR) has become an increasingly enormous worldwide health burden [1]. One of the most important reasons for this increase is inappropriately prescribed antibiotics, which are especially prevalent in children. In some developed countries, 65-67% of antibiotics used to treat pediatric patients are reported to be ineffective and not the right choice. In 2017, the World Health Organization (WHO) published a list of drug-resistant bacteria (Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli,

etc.). The aforementioned report suggested exploring new treatment options to reduce the number of deaths from drug-resistant bacteria [2]. Polymyxin B and E (or colistin) is a last-line drug effective in the treatment of infections caused by drug-resistant gram-negative bacteria (Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumonia, etc.). [1].

Colistin (COL) interacts with anionic lipopolysaccharides, displacing and neutralizing divalent cations (Ca and Mg) in the membrane of gram-negative bacteria (GNB). COL has an enormous molecular weight cannot easily pass through cell membranes, and is primarily distributed in the extracellular space. The pharmacokinetics of COL are very variable and have a narrow therapeutic window. In the 1980s, the clinical use of COL was abandoned due to its heavy side effects, but since the drug was effective in AMR, dose adjustment was prioritized to reduce side effects instead of banning it [3]. High doses cause undesirable clinical side effects, including pulmonary, neuro, and nephrotoxicity [1]. COL activates caspases 3, 8, and 9. Activation of caspases can potentially be triggered by two interacting pathways, the mitochondrial pathway (intrinsic) and the cell death receptor pathway (extrinsic). Concentration and time-dependent activation of all three caspases by COL in lung cells suggests that both the death receptor and mitochondrial pathways play a role in COL-induced apoptosis. [4]. Since 1960, there have been numerous reports of COL-induced acute respiratory failure and respiratory paralysis [5]. Direct delivery of COL to the lung is a promising strategy for pulmonary infection treatment, but high localized levels can cause pulmonary toxicity [6]. Ahmed et al. [4] reported that the drug accumulated at high levels in A549 human lung epithelial cells and caused apoptotis.

Flavonoids are natural compounds with many biological and pharmacological activities such as anti-inflammatory, antiotophagic, and antiapoptotic, especially antioxidant properties. Research on the therapeutic effects of flavonoids against toxic damaging agents has been intensified [7, 8]. Flavonoids show antioxidant properties significantly by eliminating reactive oxygen species (ROS) and reactive nitrogen species and also by increasing antioxidant enzyme capacity [9]. Rutin (RUT) is a flavone derivative composed of the flavonol quercetin and the disaccharide rutinose and has the basic activities of flavonoids [10].

RUT is a significant flavonoid with four hydroxyl groups and the routineose molecule in its structure, and these play a significant role in its biological activity. RUT is found in citrus fruits such as grapefruit, orange, lemon, and fruits and vegetables such as spinach, onion, and apple. In particular, the presence of routineose increases the number of active sites of RUT, making it a more effective molecule [9,10].

In the presented study, the potential effects of RUT on Sprague Dawley rats with lung injury induced by COL were examined in terms of oxidative stress, inflammation, apoptosis and autophagic pathway markers and possible damage mechanisms were tried to be revealed.

2. MATERIAL AND METHODS

2.1. Drug and Chemicals

COL (Colimycin® 150 mg/vial, Koçak Pharma, Istanbul, Turkey) was obtained from a local pharmacy. RUT (≥ 94%) and other chemicals were of analytical purity and purchased from Sigma Chemical Co. (St. Louis, MO, USA). ELISA kits for Nuclear factor kappa B (NF-κB) and Tumor necrosis factor alpha (TNF-α) were obtained from YL Biont (Shangai, China); Interleukin 1 beta (IL-1β), Myeloperoxidase (MPO), Cyclooxygenase-2 (COX-2), Mammalian target of rapamycin (mTOR), Cysteine aspartate specific protease-3 (caspase-3) and Beclin-1 were obtained from Sunred Biological Technology Company (Shangai, China).

2.2. Experimental Animals and Ethics Committee Aprproval

Thirty-five male Sprague Dawley rats from Atatürk University Animal Experimentation Center were used. Animals were kept in clean cages in a controlled room with a constant temperature of 24-25 °C and a 12-hour dark-light cycle. They were provided with unlimited access to water and standard chow. After the rats were allowed to rest in their cages for one week and adapted to the environment, the experiment was started. The ethics committee approval of the study was obtained from the Atatürk University Animal Experiments Local Ethics Committee with meeting number 2024/02 and decision number 34 dated 26.02.2024.

2.3. Experimental Design

In the dose selection of COL and RUT used in the study, Çelik et al. [11] were taken as a reference.

The rats were divided into five groups with seven rats in each group.

1. Control: 7 days oral and intraperitoneal (i.p.) saline was given.
2. RUT: 100 mg/kg RUT was given orally for seven days.
3. COL: 15 mg/kg COL was given i.p. for seven days.
4. COL+RUT50: 15 mg/kg COL was given i.p. for seven days. 50 mg/kg RUT was given orally for seven days 30 minutes after COL administration.
5. COL+RUT100: 15 mg/kg COL was given i.p. for seven days. 100 mg/kg RUT was given orally for seven days 30 minutes after COL administration.

Twenty-four hours after the last administration (day 8), the rats were decapitated under mild sevoflurane anesthesia, and lung tissue was removed and stored at -20oC until biochemical analyses were performed.

2.4. Evaluation of Oxidative Stress

Lung tissue was ground using liquid nitrogen (Tissue Lyser II, Qiagen). The lung tissues were homogenized in a 1:10 (weight/volume) ratio of tissue and 1.15% potassium chloride buffer. A portion of the homogenate was centrifuged at 10,000 rpm for 20 minutes at 4°C and the supernatant was used to measure glutathione peroxidase (GPx) activity and glutathione (GSH) level. The remaining homogenate was centrifuged at 3500 rpm for 15 minutes, and the supernatants were used for catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) analysis. Measurements were performed as follows; CAT activity was determined by Aebi [12], GPx activity by Matkovics [13], SOD activity by Sun et al [14], MDA level by Placer et al [15], and GSH level by Sedlak and Lindsay [16]. The total protein in the homogenate was determined according to the Lowry et al. [17] method.

2.5. Determination of Lung Tissue NF-κB, TNF-α, IL-1β, MPO, COX-2, mTOR, Beclin-1 and Caspase-3 Levels by ELISA Method

The supernatants were prepared by centrifugation at 3500 rpm for 15 minutes and analyzed using the ELISA kit according to the manufacturer's protocol. 100 mg of ground lung tissue was diluted 1:20 with phosphate buffer (0.1 M, pH 7.4). The results were calculated using the standard graphs of the kits from the absorbance values obtained by reading the ELISA plates in a microplate reader (Bio-Tek, Winooski, VT, USA).

2.6. Statistical Analysis

One-way analysis of variance (ANOVA) and Tukey post hoc test (version 20.0; SPSS, Chicago, IL) was used to determine the difference between the groups and the significance levels. $p<0.05$ was considered a significant difference. All values were expressed as the mean ± standard error of the mean (SEM).

3. RESULTS

3.1. MDA Levels

When lung tissue MDA levels were examined (Figure 1A), no difference was found between the control and RUT groups ($p>0.05$), MDA values increased in the COL group compared to the control group ($p<0.0001$), and 50 and 100 doses of RUT given together with COL were found to be effective in reducing MDA values ($p<0.0001$).

3.2. GSH Levels

It was found that GSH levels in the COL group decreased compared to the control group ($p<0.0001$), there was no difference between the control and RUT groups ($p>0.05$), and both doses of RUT given together with COL were effective in increasing GSH levels ($p<0.0001$) (Figure 1B).

3.3. GPx Activities

While there was no difference between control and RUT group GPx activities ($p>0.05$), GPx activities in COL group decreased compared to control and RUT groups ($p<0.0001$), GPx activities in COL+RUT50 and COL+RUT100 groups increased compared to COL group ($p<0.0001$) (Figure 1C).

3.4. SOD Activities

SOD activities in the COL group decreased compared to the control and RUT groups ($p<0.0001$), while both doses of RUT treatment increased these activities compared to the COL group ($p<0.0001$) (Figure 1D).

3.5. CAT Activities

When lung tissue CAT activities were examined (Figure 1E), it was found that there was no difference between the control and RUT groups ($p>0.05$), CAT activities in the COL group decreased compared to the control group ($p<0.0001$), RUT 50 doses administered together with COL was not effective in increasing the activity ($p>0.05$), and RUT 100 doses increased CAT activity ($p<0.01$).

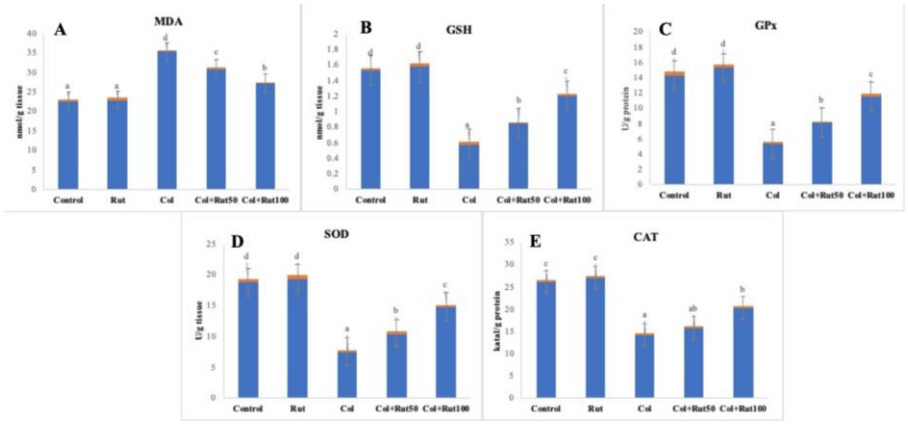

Figure 1. MDA (A), GSH (B) levels and GPx (C), SOD (D), CAT (E) activities in lung tissue after COL and RUT treatments. Different letters (a, b, c, d, e) indicate differences between groups ($p<0.05$).

3.6. NF-κB Levels

COL group NF-κB levels increased ($p<0.001$) when compared with control and RUT groups, and RUT 50 and 100 doses were effective in decreasing NF-□B levels ($p<0.0001$) (Figure 2A).

3.7. TNF-α Levels

While there was no difference between the control and RUT groups ($p>0.05$), TNF-α levels increased in the COL group compared to the control group ($p<0.0001$), and 50 and 100 doses of RUT given together with COL were effective in reducing TNF-α levels ($p<0.0001$) (Figure 2B).

3.8. IL-1β Levels

When lung tissue IL-1β levels were examined (Figure 2C), it was determined that there was no difference between the control group and the RUT group ($p>0.05$), and IL-1β levels in the COL group increased compared to the control and RUT groups ($p<0.0001$), both doses of RUT were effective and decreased IL-1β levels ($p<0.0001$).

3.9. MPO Activities

MPO activity in the COL group increased compared to the control and RUT group ($p<0.0001$), there was no difference between the control and RUT group ($p>0.05$), and RUT 50 and 100 doses were effective in decreasing the activity ($p<0.0001$) (Figure 2D).

3.10. COX-2 Activities

While there was no difference between the COX-2 activities of the control and RUT groups ($p>0.05$), it was determined that COX-2 activity increased in the COL group compared to the control group ($p<0.0001$), while COX-2 activity decreased with RUT 50 and 100 applications ($p<0.0001$) (Figure 2E).

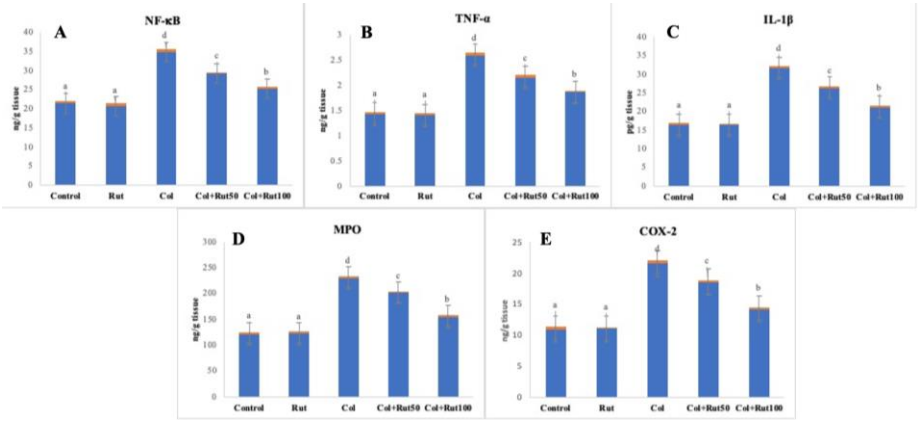

Figure 2. NF-κB (A), TNF-α (B), IL-1β (C) levels and MPO (D), COX-2 (E) activities in lung tissue after COL and RUT treatments. Different letters (a, b, c, d, e) indicate differences between groups ($p<0.05$).

3.11. mTOR Levels

When lung tissue mTOR levels were examined (Figure 3A), it was found that mTOR level decreased in COL-treated rats compared to the control and RUT groups ($p<0.0001$), and RUT 50 and 100 doses were effective in increasing mTOR levels ($p<0.0001$).

3.13. Beclin-1 Levels

When Beclin-1 levels were analyzed (Figure 3B.), it was found that Beclin-1 level increased in the COL group compared to the control group ($p<0.0001$), and RUT 50 and 100 doses administered together with COL were effective in reducing Beclin-1 levels ($p<0.0001$).

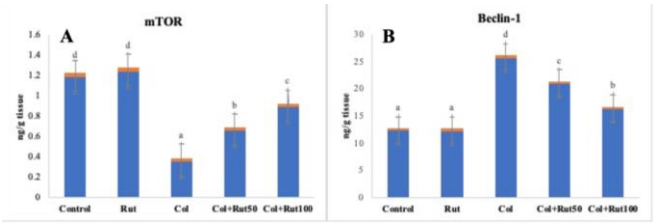

Figure 3. mTOR (A) and Beclin-1 (B) levels in lung tissue after COL and RUT treatments. Different letters (a, b, c, d, e) indicate differences between groups ($p<0.05$).

3.14. Caspase-3 activities

When Caspase-3 activity was examined (Figure 4), it was determined that there was no difference between the control group and the RUT group ($p>0.05$), the Caspase-3 activity in the COL group increased compared to the control and RUT groups ($p<0.0001$), both doses of RUT were effective in reducing Caspase-3 activity ($p<0.0001$), and there was no difference between RUT 50 and 100 doses in terms of effect ($p>0.05$).

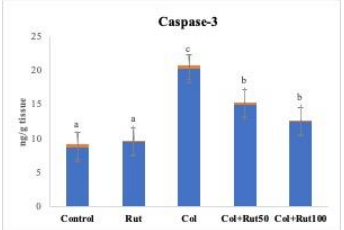

Figure 4. Caspase-3 activity in lung tissue after COL and RUT treatments. Different letters (a, b, c, d, e) indicate differences between groups ($p<0.05$).

4. DISCUSSION AND CONCLUSION

COL (polymyxin E) is a glycopeptide antibiotic approved for medical use and used as a last resort in patients. It is commercially available in the form of COL sulfate for topical and oral use and colistimethate sodium for parenteral and inhalation use [18]. COL, a cationic polypeptide, has a bactericidal effect on multidrug-resistant GNB and is still one of the few options for the treatment of infections caused by GNB. COL is often used to treat GNB infections by intramuscular injection. In-vivo and in-vitro studies have demonstrated that COL use causes pulmonary toxicity, but the molecular mechanism underlying this toxicity has not been fully elucidated [1]. Therefore, in this study, the effects of RUT were investigated by oxidative stress, inflammation, apoptotic, and autophagic markers in rats with COL-induced lung injury.

Oxidative stress is recognized as one of the most important causes of tissue damage. Excessive production of ROS due to different reasons causes a decrease in antioxidant capacity, and this situation manifests itself as oxidative stress [19-21]. ROS produced in excessive amounts shape cell damage by affecting many macromolecules, especially proteins and lipids, one of the most significant components of the cell membrane [22, 23]. MDA, the end product of lipid peroxidation, is the most important marker of oxidative stress, and oxidative stress activates various defense systems in cells [24-26].

Antioxidants interact with unstable molecules such as ROS to stabilize them and prevent cell damage [27-29]. SOD, CAT, and GPx are at the base of the cellular antioxidant defense line. SOD is responsible for the scavenging of superoxide radicals, while CAT and GPx are responsible for the decomposition of H2O2 into water and molecular oxygen. GSH, apart from being the substrate of GPx, also helps to maintain the redox state in cells [30, 31]. Studies have shown that COL increases MDA levels and causes cell damage by decreasing SOD, CAT, GPx activities, and GSH levels [1, 11, 32]. In the present study, it was determined that MDA levels increased, SOD, CAT, GPx activities and GSH levels decreased and oxidative stress developed in the cell of rats administered COL. It was determined that 50 and 100 doses of RUT administered together with COL protected the cell membrane and integrity by decreasing MDA levels, increased SOD, CAT, GPx activities, and GSH levels, and protected the cell from damage caused by oxidative stress by strengthening the antioxidant defense system. Aktaş et al. [33] reported that RUT decreased MDA levels by increasing antioxidant enzyme activities in lung damage and protected the cell from the effects of oxidative stress.

Inflammation, another damage pathway triggered by oxidative stress, is an important mechanism that causes an increase in lung damage. Increased oxidative stress in lung cells also increases the release of inflammatory cytokines and chemokines from the lung cells [34]. NF-κB is one of the transcription factors effective in inflammatory damage and regulates the release of pro-inflammatory cytokines (such as TNF-α, IL-1β, and COX-2) [35-37]. ROS have been shown to play a role in the activation of pro-inflammatory mediators NF-□B and TNF-α, which promote tissue inflammation, and it has been reported that suppression of NF-□B will decrease TNF-α and suppress inflammation [37]. In a study conducted with different toxic agents, it was reported that inflammatory cytokines in lung tissue increased via ROS [38].

MPO, one of the enzymes that is effective in inflammation and oxidative stress damage at the cellular level, is a heme protein released by leukocytes. Its level is considered a reliable biomarker and indicates neutrophil infiltration when it increases [39]. In the present study, COL administration increased inflammation in lung tissue by causing an increase in NF-κB, TNF- α, IL -1β, COX-2, and MPO levels, while both doses of RUT administered together with COL suppressed TNF- α, IL -1β, COX-2 and MPO production and decreased inflammation by suppressing NF-κB release. In a study on rats, it was revealed that COL caused inflammation by increasing the levels of NF-κB, TNF- α, and IL -1β [40]. It has been reported that RUT has anti-inflammatory properties and reduces inflammation increased by chemical agents by suppressing cytokine production [7, 9, 41].

mTOR is a primary regulator with significant functions in autophagy and provides negative regulation of autophagy. mTOR is a serine/threonine kinase that is one of the major regulators of cellular functions such as growth, proliferation, and survival. While regulating cellular functions, mTOR; regulates cellular activities such as protein synthesis, energy metabolism, and stress response by bringing together different signaling pathways. [42]. Studies have shown that COL suppresses mTOR activity and accelerates autophagy [1]. In the present study, it was determined that mTOR activity decreased in COL-treated rats, while COL and RUT administration caused an increase in activity. It has been reported in different studies that RUT regulates mTOR activity and seriously increases dose-dependent mTOR levels [43, 44].

Autophagy is a program that manifests itself in situations such as nutrient, growth factor deficiency, and stress and is effective in cellular homeostasis, metabolism, and transport of material from the cytoplasm to lysosomes. Autophagy also allows cells to recycle nutrients, maintain cell energy balance, and break down toxic cytoplasmic components [45]. Beclin-1 is one of the most significant indicators interested in the autophagic process and determines the extent of autophagic damage [9]. A study revealed that COL accelerates autophagy by increasing Beclin-1 levels in lung tissue [1]. COL was also found to increase Beclin-1 levels in different studies on rats [2, 45]. In the present study, it was determined that COL administration accelerated autophagy by increasing Beclin-1 levels in rat lung tissue, while RUT administration suppressed autophagy by decreasing Beclin-1 levels. Studies have shown that rutin exhibits

anti-autophagic properties in organ toxicity models developed with different chemicals, suppresses the expression of Beclin-1, the most important marker of the autophagic pathway, and is effective in protecting the cell from autophagy [7, 9, 10].

Apoptosis, also known as programmed cell death, is a normal process for the elimination of unwanted cells and maintenance of tissue homeostasis [46, 47]. Caspase-3, one of the most significant markers of apoptosis, is a death protease that is often induced because it increases the specific cleavage of many cellular proteins and leads to DNA fragmentation, one of the characteristic cellular changes of apoptosis [48-50]. Cytokines such as TNF-α have also been reported to play a role in triggering apoptosis [51]. It has been reported in different studies that COL accelerates caspase-3 activity by increasing it [52, 53]. In the present study, it was found that caspase-3 activity increased and apoptosis accelerated in the lung tissue of COL-treated rats, and both doses of RUT suppressed apoptosis by decreasing this activity. It was reported by Gür and Kandemir [54] that RUT suppressed apoptosis by decreasing caspase-3 activity in lung tissue. In toxicity models developed with different chemicals in different organs, it has been reported that rutin exhibits antiapoptotic properties, suppresses the apoptotic pathway, especially Caspase-3, and protects the cell from apoptosis [55- 58].

As a conclusion, it was determined that COL triggered oxidative stress by increasing MDA level and causing a decrease in antioxidant enzyme activities and the increase in oxidative stress increased apoptosis and autophagy, especially inflammation, while RUT application showed the opposite effect, and tried to protect the cell. In light of the findings obtained, it is thought that the use of RUT in COL-induced lung damage will be beneficial.

REFERENCES

[1] Dai C, Li M, Sun T, Zhang Y, Wang Y, Shen Z, et al. Colistin-induced pulmonary toxicity involves the activation of NOX4/TGF-β/mtROS pathway and the inhibition of Akt/mTOR pathway. Food Chem Toxicol. 2022;163:112966.

[2] Kankılıç NA, Şimşek H, Akaras N, Gür C, İleritürk M, Küçükler S, et al. Protective effects of naringin on colistin-induced damage in rat testicular tissue: Modulating the levels of Nrf-2/HO-1, AKT-2/FOXO1A, Bax/Bcl2/Caspase-3, and Beclin-1/LC3A/LC3B signaling pathways. J Biochem Mol Toxicol. 2024;38(2):e23643.

[3] Aksu EH, Kandemir FM, Küçükler S. The effects of hesperidin on colistin-induced reproductive damage, autophagy, and apoptosis by reducing oxidative stress. Andrologia. 2021;53(2):e13900.

[4] Ahmed MU, Velkov T, Lin YW, Yun B, Nowell CJ, Zhou F, Zhou QT, Chan K, Azad MAK, Li J. Potential Toxicity of Polymyxins in Human Lung Epithelial Cells. Antimicrob Agents Chemother. 2017;61(6):e02690-16.

[5] Shrestha A, Soriano SM, Song M, Chihara S. Intravenous colistin-induced acute respiratory failure: A case report and a review of literature. Int J Crit Illn Inj Sci. 2014;4(3):266-70.

[6] Velkov T, Abdul Rahim N, Zhou QT, Chan HK, Li J. Inhaled anti-infective chemotherapy for respiratory tract infections: successes, challenges and the road ahead. Adv Drug Deliv Rev. 2015;85:65-82.

[7] Gur C, Kandemir FM. Molecular and biochemical investigation of the protective effects of rutin against liver and kidney toxicity caused by malathion administration in a rat model. Environ Toxicol. 2023;38(3):555-565.

[8] Simsek H, Akaras N. Acacetin ameliorates acetylsalicylic acid-induced gastric ulcer in rats by interfering with oxidative stress, inflammation, and apoptosis. Int J Med Biochem 2023;6(2):96-103.

[9] Kandemir FM, Ileriturk M, Gur C. Rutin protects rat liver and kidney from sodium valproate-induce damage by attenuating oxidative stress, ER stress, inflammation, apoptosis and autophagy. Mol Biol Rep. 2022;49(7):6063-6074.

[10] Küçükler S, Kandemir FM, Özdemir S, Çomaklı S, Caglayan C. Protective effects of rutin against deltamethrin-induced hepatotoxicity and nephrotoxicity in rats via regulation of oxidative stress, inflammation, and apoptosis. Environ Sci Pollut Res Int. 2021;28(44):62975-62990.

[11] Çelik H, Kandemir FM, Caglayan C, Özdemir S, Çomaklı S, Kucukler S, Yardım A. Neuroprotective effect of rutin against colistin-induced oxidative stress, inflammation and apoptosis in rat brain associated with the CREB/BDNF expressions. Mol Biol Rep. 2020;47(3):2023-2034.

[12] Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6.

[13] Matkovics B. Determination of enzyme activity in lipid peroxidation and glutathione pathways. Laboratoriumi Diagnosztika. 1988;15:248-250.

[14] Sun YI, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clinical chemistry, 1988;34(3):497-500.

[15] Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analytical biochemistry. 1966;16(2):359-64.

[16] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry, 1968;25:192-205.

[17] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951;193:265–75.

[18] Nasrullah MZ, Eljaaly K, Neamatallah T, et al. Omeprazole Prevents Colistin-Induced Nephrotoxicity in Rats: Emphasis on Oxidative Stress, Inflammation, Apoptosis and Colistin Accumulation in Kidneys [published correction appears in Pharmaceuticals (Basel). 2024;17(4):540.

[19] Kandemir FM, Ozkaraca M, Küçükler S, Caglayan C, Hanedan B. Preventive effects of hesperidin on

diabetic nephropathy induced by streptozotocin via modulating TGF-β1 and oxidative DNA damage. Toxin reviews. 2018;37(4):287-93.
[20] Ekinci Akdemir FN, Yildirim S, Kandemir FM, Aksu EH, Guler MC, Kiziltunc Ozmen H, et al. The antiapoptotic and antioxidant effects of eugenol against cisplatin-induced testicular damage in the experimental model. Andrologia. 2019;51(9):e13353.
[21] Zeren S, Bayhan Z, Kocak FE, Kocak C, Akcılar R, Bayat Z, et al. Gastroprotective effects of sulforaphane and thymoquinone against acetylsalicylic acid-induced gastric ulcer in rats. J Surg Res. 2016;203(2):348-59.
[22] Kocak C, Kocak FE, Akcilar R, Isiklar OO, Kocak H, Bayat Z, et al. Molecular and biochemical evidence on the protective effects of embelin and carnosic acid in isoproterenol-induced acute myocardial injury in rats. Life Sci. 2016;147:15-23.
[23] Kankılıç NA, Şimşek H, Akaras N, Gür C, Küçükler S, İleritürk M, et al. The ameliorative effects of chrysin on bortezomib-induced nephrotoxicity in rats: Reduces oxidative stress, endoplasmic reticulum stress, inflammation damage, apoptotic and autophagic death. Food Chem Toxicol. 2024;190:114791.
[24] Şimşek H, Akaras N, Gür C, Küçükler S, Kandemir FM. Beneficial effects of Chrysin on Cadmium-induced nephrotoxicity in rats: Modulating the levels of Nrf2/HO-1, RAGE/NLRP3, and Caspase-3/Bax/Bcl-2 signaling pathways. Gene. 2023;875:147502.
[25] Akaras N, Ileriturk M, Gur C, Kucukler S, Oz M, Kandemir FM. The protective effects of chrysin on cadmium-induced pulmonary toxicity; a multi-biomarker approach. Environ Sci Pollut Res Int. 2023;30(38):89479-89494.
[26] Keleş O, Can S, Cigsar G, Colak S, Erol H, Akaras N, et al. Hepatoprotective effects of B-1, 3-(D)-glucan on bortezomib-induced liver damage in rats. Kafkas Universitesi Veteriner Fakultesi Dergisi, 2014;20(6):929-38.
[27] Ileriturk M, Ileriturk D, Kandemir O, Akaras N, Simsek H, Erdogan E, et al. Naringin attenuates oxaliplatin-induced nephrotoxicity and hepatotoxicity: A molecular, biochemical, and histopathological approach in a rat model. J Biochem Mol Toxicol. 2024;38(1):e23604.
[28] Şimşek H, Gür C, Küçükler S, İleritürk M, Akaras N, Öz M, et al. Carvacrol Reduces Mercuric Chloride-Induced Testicular Toxicity by Regulating Oxidative Stress, Inflammation, Apoptosis, Autophagy, and Histopathological Changes. Biol Trace Elem Res. 2023 Dec 22.
[29] Ozyigit F, Deger AN, Kocak FE, Ekici MF, Simsek H, Arık O. Protective effects of hesperidin in gastric damage caused by experimental ischemia-reperfusion injury model in rats. Acta Cir Bras. 2024;39:e391124.
[30] Gur C, Akarsu SA, Akaras N, Tuncer SC, Kandemir FM. Carvacrol reduces abnormal and dead sperm counts by attenuating sodium arsenite-induced oxidative stress, inflammation, apoptosis, and autophagy in the testicular tissues of rats. Environ Toxicol. 2023;38(6):1265-1276.
[31] Aydin M, Cevik A, Kandemir FM, Yuksel M, Apaydin AM. Evaluation of hormonal change, biochemical parameters, and histopathological status of uterus in rats exposed to 50-Hz electromagnetic field. Toxicol Ind Health. 2009;25(3):153-8.
[32] Hanedan B, Ozkaraca M, Kirbas A, Kandemir FM, Aktas MS, Kilic K, et al. Investigation of the effects of hesperidin and chrysin on renal injury induced by colistin in rats. Biomed Pharmacother. 2018;108:1607-1616.
[33] Aktas M, Kandemir F, Özkaraca M, Hanedan B, Kirbas A. Protective Effects of Rutin on Acute Lung Injury Induced by Oleic Acid in Rats. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2017;23(3):445-51.
[34] Yesildag K, Gur C, Ileriturk M, Kandemir FM. Evaluation of oxidative stress, inflammation, apoptosis, oxidative DNA damage and metalloproteinases in the lungs of rats treated with cadmium and carvacrol. Mol Biol Rep. 2022;49(2):1201-1211.
[35] Semis HS, Gur C, Ileriturk M, Kandemir FM, Kaynar O. Evaluation of Therapeutic Effects of Quercetin Against Achilles Tendinopathy in Rats via Oxidative Stress, Inflammation, Apoptosis, Autophagy, and Metalloproteinases. Am J Sports Med. 2022;50(2):486-498.
[36] Şimşek H, Küçükler S, Gür C, Akaras N, Kandemir FM. Protective effects of sinapic acid against lead acetate-induced nephrotoxicity: a multi-biomarker approach. Environ Sci Pollut Res Int. 2023;30(45):101208-22.
[37] Ileriturk M, Kandemir O, Akaras N, Simsek H, Genc A, Kandemir FM. Hesperidin has a protective effect on paclitaxel-induced testicular toxicity through regulating oxidative stress, apoptosis, inflammation and endoplasmic reticulum stress. Reprod Toxicol. 2023;118:108369.
[38] Şimşek H, Küçükler S, Gür C, İleritürk M, Aygörmez S, Kandemir FM. Protective effects of zingerone against sodium arsenite-induced lung toxicity: A multi-biomarker approach. Iran J Basic Med Sci. 2023;26(9):1098-1106.
[39] Caglayan C, Kandemir FM, Yildirim S, Kucukler S, Eser G. Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level, oxidative stress, apoptosis and inflammation in rats. J Trace Elem Med Biol. 2019;54:69-78.
[40] Yılmaz S, Küçükler S, Şimşek H, Aygörmez S, Kandemir FM. Naringin protects against colistin-induced sciatic nerve damage by reducing oxidative stress, apoptosis and inflammation damage. J. Exp. Clin. Med. 2024;41(1):53-9.
[41] He Q, Hao H, Zhao K. (2024). Investigating the anti-inflammatory effects of rutin in carbon tetrachloride-induced hepatotoxicity: role of TLR4/MyD88/NFκB signaling pathway modulation. Pharmacognosy Magazine. 2024;20(1):107-15.

[42] Wang Y, Liu Z, Shu S, Cai J, Tang C, Dong Z. AMPK/mTOR Signaling in Autophagy Regulation During Cisplatin-Induced Acute Kidney Injury. Front Physiol. 2020;11:619730.
[43] Fei J, Sun Y, Duan Y, Xia J, Yu S, Ouyang P, et al. Low concentration of rutin treatment might alleviate the cardiotoxicity effect of pirarubicin on cardiomyocytes via activation of PI3K/AKT/mTOR signaling pathway. Biosci Rep. 2019;39(6):BSR20190546.
[44] Wu X, Wang J, Li B, Gong M, Cao C, Song L, et al. Chlorogenic acid, rutin, and quercetin from Lysimachia christinae alleviate triptolide-induced multi-organ injury in vivo by modulating immunity and AKT/mTOR signal pathway to inhibit ferroptosis and apoptosis. Toxicol Appl Pharmacol. 2023;467:116479.
[45] Lu Z, Miao Y, Muhammad I, Tian E, Hu W, Wang J, et al. Colistin-induced autophagy and apoptosis involves the JNK-Bcl2-Bax signaling pathway and JNK-p53-ROS positive feedback loop in PC-12 cells. Chem Biol Interact. 2017;277:62-73.
[46] Akarsu SA, Gür C, İleritürk M, Akaras N, Küçükler S, Kandemir FM. Effect of syringic acid on oxidative stress, autophagy, apoptosis, inflammation pathways against testicular damage induced by lead acetate. J Trace Elem Med Biol. 2023;80:127315.
[47] Akcılar R, Akcılar A, Koçak C, Koçak FE, Bayat Z, Şimşek H, et al. Effects of Ukrain on intestinal apoptosis caused by ischemia-reperfusion injury in rats. Int J Clin Exp Med. 2015;8(12):22158-66.
[48] Şimşek H, Demiryürek Ş, Demir T, Atabay HD, Çeribasi AO, Bayraktar R, et al. Assessment of expressions of Bcl-XL, b-FGF, Bmp-2, Caspase-3, PDGFR-α, Smad1 and TGF-β1 genes in a rat model of lung ischemia/reperfusion. Iran J Basic Med Sci. 2016;19(2):209-14.
[49] Semis HS, Kandemir FM, Kaynar O, Dogan T, Arikan SM. The protective effects of hesperidin against paclitaxel-induced peripheral neuropathy in rats. Life Sci. 2021;287:120104.
[50] Gur C, Kandemir FM, Caglayan C, Satıcı E. Chemopreventive effects of hesperidin against paclitaxel-induced hepatotoxicity and nephrotoxicity via amendment of Nrf2/HO-1 and caspase-3/Bax/Bcl-2 signaling pathways. Chem Biol Interact. 2022;365:110073.
[51] Güçlü A, Erken HA, Erken G, Dodurga Y, Yay A, Özçoban Ö, et al. The effects of ozone therapy on caspase pathways, TNF-α, and HIF-1α in diabetic nephropathy. Int Urol Nephrol. 2016;48(3):441-50.
[52] Worakajit N, Thipboonchoo N, Chaturongakul S, Jutabha P, Soontornniyomkij V, Tuchinda P, et al. Nephroprotective potential of Panduratin A against colistin-induced renal injury via attenuating mitochondrial dysfunction and cell apoptosis. Biomed Pharmacother. 2022;148:112732.
[53] Aksu EH, Kandemir FM, Küçükler S, Mahamadu A. Improvement in colistin-induced reproductive damage, apoptosis, and autophagy in testes via reducing oxidative stress by chrysin. J Biochem Mol Toxicol. 2018;32(11):e22201.
[54] Gür C, Kandemir FM. Evaluation of the levels of metalloproteinases as well as markers of oxidative stress and apoptosis in lung tissues after malathion and rutin administrations to rats. TJNS. 2022;11(3):51-7.
[55] Rafiee S, Nouri A, Heidarian E. Role of NF-κB/IL-1β Pathway and Caspase 3 in mediating the hepatoprotective effect of rutin against paraquat-ınduced liver toxicity in male rats. Chem Biodivers. 2023;20(4):e202200248.
[56] Rahmani S, Naraki K, Roohbakhsh A, Hayes AW, Karimi G. The protective effects of rutin on the liver, kidneys, and heart by counteracting organ toxicity caused by synthetic and natural compounds. Food Sci Nutr. 2022;11(1):39-56.
[57] Akaras N, Kandemir FM, Şimşek H, Gür C, Aygörmez S. Antioxidant, Antiinflammatory, and Antiapoptotic Effects of Rutin in Spleen Toxicity Induced by Sodium Valproate in Rats. TJNS. 2023;12(2), 138-144.
[58] Caglayan C, Kandemir FM, Darendelioğlu E, Yıldırım S, Kucukler S, Dortbudak MB. Rutin ameliorates mercuric chloride-induced hepatotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. J Trace Elem Med Biol. 2019; 56, 60-68.