Araştırma Makalesi https://doi.org/10.46810/tdfd.1132497 Research Article

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

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

Cihan GÜR[1*], Fatih Mehmet KANDEMİR[2]

[1] Atatürk University, Veterinary Faculty, Biochemistry Department, Erzurum, Türkiye
[2] Aksaray University, Medicine Faculty, Medical Biochemistry Department, Aksaray, Türkiye
Cihan GÜR ORCID No: 0000-0001-6775-7858
Fatih Mehmet KANDEMİR ORCID No: 0000-0002-8490-2479

**Corresponding author: cihan.gur@atauni.edu.tr*

(Received: 17.06.2022, Accepted: 02.08.2022, Online Publication: 29.09.2022)

Keywords
Apoptosis,
Lung,
Malathion,
Metalloproteinases,
Oxidative stress,
Rutin

Abstract: Malathion (MLT) is an important environmental pollutant in the organophosphate class. Rutin (RUT), on the other hand, is one of the flavonoid family members whose effectiveness against various toxic agents has been extensively studied. In the present study, the effects of MLT and RUT treatments on oxidative stress, apoptosis and metalloproteinases in lung tissues of rats were investigated. In this study, MDA, GSH, Nrf2, HO-1, MMP2, MMP9 and caspase-3 levels in lung tissues were analyzed by biochemical or RT-PCR method after rats received MLT and/or RUT treatment for 28 days. The data showed that MLT-induced MDA levels decreased after RUT treatment. Also, it was determined that Nrf2 and HO-1 mRNA transcript levels and GSH levels suppressed by MLT approached the control group levels after RUT treatment. MLT up-regulated the expression of metalloproteinases (MMP2 and MMP9) in lung tissues, while RUT down-regulated the expression of these genes. In addition, it was observed that MLT triggered caspase-3 expression, while RUT exerted an anti-apoptotic effect by suppressing caspase-3. As a result, it was determined that while MLT showed toxic effects in the lung tissues of rats through oxidative stress, apoptosis and metalloproteinases, RUT could alleviate these toxic effects.

Ratlara Malathion ve Rutin Uygulamaları Sonrası Akciğer Dokularında Metalloproteinaz Düzeyleri ile Oksidatif Stres ve Apoptoz Belirteçlerinin Değerlendirilmesi

Anahtar Kelimeler
Akciğer,
Apoptoz, Malathion,
Metalloproteinazlar,
Oksidatif stres,
Rutin

Öz: Malathion (MLT), organofosfat sınıfında yer alan önemli bir çevresel kirleticidir. Rutin (RUT) ise çeşitli toksik ajanlara karşı etkinliği yoğun olarak araştırılan flavonoid aile üyelerinden biridir. Bu çalışmada, MLT ve RUT tedavilerinin sıçanların akciğer dokularında oksidatif stres, apoptoz ve metalloproteinazlar üzerine etkileri araştırıldı. Çalışmada, sıçanlara 28 gün boyunca MLT ve/veya RUT tedavisi verildikten sonra akciğer dokularındaki MDA, GSH, Nrf2, HO-1, MMP2, MMP9 ve kaspaz-3 seviyeleri biyokimyasal veya RT-PCR yöntemi ile analiz edildi. Veriler, MLT ile indüklenen MDA seviyelerinin RUT tedavisinden sonra düştüğünü gösterdi. Ayrıca Nrf2 ve HO-1 mRNA transkript düzeyleri ile MLT tarafından baskılanan GSH düzeylerinin RUT tedavisi sonrası kontrol grubu düzeylerine yaklaştığı belirlendi. MLT, akciğer dokularında metalloproteinazların (MMP2 ve MMP9) ekspresyonunu yukarı doğru düzenlerken, RUT bu genlerin ekspresyonunu aşağı regüle etti. Ayrıca MLT'nin kaspaz-3 ekspresyonunu tetiklediği, RUT'nin ise kaspaz-3'ü baskılayarak anti-apoptotik etki gösterdiği gözlendi. Sonuç olarak, MLT'nin oksidatif stres, apoptoz ve metalloproteinazlar yoluyla sıçanların akciğer dokularında toksik etkiler gösterirken, RUT'nin bu toksik etkileri azaltabileceği belirlendi.

1. INTRODUCTION

Pesticides are defined as chemicals used in agriculture to control weeds, insects and many other pests [1]. 1.8 billion people use 4.6 million tons of pesticides annually, and only less than 5% of these chemicals reach the target organism [2, 3]. Among the reasons for the widespread use of pesticides are the limited arable land and increasing production demand. Additionally, pesticide use becomes inevitable with new pesticide classes released every year to break pesticide resistance. Pesticides are very harmful compounds because they remain in the environment for a long time and have limited decomposition tendency [2].

Organophosphate insecticides (OPIs) are powerful toxicants that target the nervous systems of insects and other pests [4]. OPIs mainly work through inhibition of acetylcholinesterase (AChE), an enzyme that breaks down acetylcholine and causes acetylcholine to accumulate at the neuronal junction. Due to their ability to produce toxicity in non-target species, including humans, through the inhibition of acetylcholinesterase, they are considered sources of serious environmental pollution and health threat [5]. Malathion (MLT) is a broad spectrum organophosphate [OP] insecticide used to control a variety of outdoor insects in both agricultural and veterinary applications [6]. MLT is widely used due to its relatively low acute toxicity compared to other OP insecticides. Extensive studies have been conducted to evaluate the potential health effects of MLT in a variety of biological models, from amphibians to mammals. MLT has been reported to induce toxicity through inhibition of AchE followed by activation of cholinergic receptors [7]. Following exposure to MLT, this chemical can be rapidly absorbed by the skin, mucous membranes, gastrointestinal tract, eyes, and respiratory system. MLT has lipophilic properties which allow to spread rapidly to other tissues and to accumulate AChE in target organs. Apart from this, it has been reported that MLT causes the formation of reactive oxygen species (ROS), weakening of the antioxidant system and, consequently, induction of oxidative stress [8]. Therefore, it is thought that the use of antioxidant compounds may have beneficial effects against MLT-induced pulmonary toxicity.

Flavonoids are potent antioxidant compounds that inhibit lipid peroxidation and platelet aggregation. These compounds directly scavenge ROS, reactive nitrogen species, protect tissue from free radicals and activate antioxidant enzymes [9]. Rutin (RUT) is a quercetin glycone with a flavonol structure. It is predominantly found in citrus fruits such as oranges, grapefruits, lemons and limes. It is a member of bioflavonoids with antioxidant, anti-inflammatory, antiallergenic, antiviral and anticarcinogenic properties [10]. Previous studies have shown that RUT can alleviate the damaging effects of various toxic drugs with its antioxidant properties [11-13]. However, we could not find a study investigating the effect of RUT against MLT-induced lung toxicity. Therefore, the protective properties of RUT against MLT-induced lung toxicity were investigated in the present study.

2. MATERIAL AND METHOD

2.1. Supply, Care and Ethics Committee Approval of Experimental Animals

Sprague Dawley rats used in the study were obtained from Atatürk University Medical Experimental Application and Research Center. Animals were 10-12 weeks old and weighed 220-250 g. The environmental conditions in which the rats were housed had a temperature of 24 ± 1°C, a humidity of 45 ± 5%, and a 12-hour light/dark cycle. The animals had access to standard pellet food and water *ad libitum* throughout the treatment. Ethics committee approval was given for the 28-day study by Atatürk University Animal Experiments Local Ethics Committee (Protocol no: 2022-7-112).

2.2. Experiment Design

In the experiment, 35 rats were divided into 5 groups, 7 in each group. The doses of MLT and RUT given to the animals were determined with reference to the previous studies. The groups are designed as given below:

1. Control Group : The animals were given saline orally for 28 days.
2. RUT 100 Group : Animals were given 100 mg/kg/body weight RUT orally for 28 days [14].
3. MLT Group : Animals were given 100 mg/kg/body weight MLT orally for 28 days [15].
4. MLT+RUT 50 Group : Animals were given 50 mg/kg/body weight RUT orally for 28 days, 30 minutes later MLT was administered orally at a dose of 100 mg/kg/body weight.
5. MLT+RUT 100 Group : Animals were given 100 mg/kg/body weight RUT orally for 28 days, 30 minutes later MLT was administered orally at a dose of 100 mg/kg/body weight.

On the 29th day of the study, the rats were decapitated under mild sevoflurane anesthesia and their lung tissues were removed. Tissues were stored at -80 °C until biochemical and molecular analysis.

2.3. Analysis of Malondialdehyde Levels in Lung Tissue

Lung tissues from rats were pulverized in liquid nitrogen by means of a tissue shredder (Tissue Lyser II, Qiagen, The Netherlands). Then the tissues were homogenized in 1.15% KCl buffer at a ratio of 1:10 (weight/volume). The obtained homogenates were centrifuged at 1000xg for 15 minutes at +4 °C. Malondialdehyde (MDA) levels in homogenates was analyzed by the method developed by Placer et al. (1966) [16]. Results are presented as nmol/g tissue.

2.4. Analysis of Glutathione Levels in Lung Tissue

For glutathione (GSH) analysis, homogenates, the preparation of which was explained in the previous

section, were centrifuged at 9000xg at +4 °C. GSH levels were determined in the supernatants obtained afterwards, using the method of Sedlak et al (1968) [17]. Obtained results are presented as nmol/g tissue.

2.5. Total RNA Isolation from Lung Tissue

Total RNA isolation from pulverized lung tissues was performed using hibrizol reagent (HibriGen). For this, 60 mg of pulverized lung tissues were weighed into sterile eppendorf tubes and 2 ml of hybrisol was added. All procedures were carried out in accordance with the manufacturer's instructions. In the last step, after washing the total RNAs with 75% ethanol, they were dissolved with RNase-free water and their concentrations were measured in the nanodrop device.

2.6. cDNA Synthesis from Total RNAs

cDNA synthesis from total RNAs was performed with the iScript™ cDNA Synthesis Kit (BIO-RAD, USA). cDNA synthesis was carried out in strict accordance with the instructions given by the manufacturer.

2.7. RT-PCR

At the RT-PCR stage, the primers of the genes whose sequences are given in Table 1, cDNAs, iTaq Universal SYBR® Green Supermix and a mixture with RNase-DNase-free water were prepared. The reaction was started by entering the temperatures and times specified in the procedure that came with iTaq Universal SYBR® Green Supermix into the ROTOR-GENE Q (Qiagen, Germany) device. At the end of the procedures, the fold changes of the relevant genes were calculated using the $2^{-deltadelatCT}$ method using the CT values obtained from the device [18]. GAPDH was used as internal control.

Table 1: Primer sequences

Gene	Sequences (5'-3')	Length (bp)
MMP2	F: CTCTAGGAGAAGGACAAGTG R: CTCAAAGTTGTACGTGGTGG	158
MMP9	F: AGCTGGCAGAGGATTACCTG R: ATGATGGTGCCACTTGAGGT	230
Nrf2	F: TTTGTAGATGACCATGAGTCGC R: TCCTGCCAAACTTGCTCCAT	161
HO-1	F: ATGTCCCAGGATTTGTCCGA R: ATGGTACAAGGAGGCCATCA	144
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270
GAPDH	F: GAGTATGTCGTGGAGTCTAC R: CAGGATGCATTGCTGACAAT	179

2.8. Statistical Analysis

In the statistical analysis of the data, one-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used in SPSS 20.0 program. Results are presented as mean ± SD. P < 0.05 was considered statistically significant.

3. RESULTS

3.1. Oxidative Stress State in Lung Tissue

After MLT and RUT treatments were applied to rats, tissue MDA and GSH levels and nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1) mRNA transcript levels were analyzed to determine the oxidative stress status in lung tissue (Figure 1). It was determined that MLT downregulated the Nrf2 and HO-1 genes, caused the depletion of GSH stores and caused a significant increase in MDA levels ($p<0.05$). On the other hand, RUT treatment provided activation in Nrf2 and HO-1 genes. In addition, RUT reduced MDA levels by regenerating GSH stores. While there was no difference between the doses of RUT on Nrf2 expression, HO-1 expression was triggered in a dose-dependent manner ($p<0.05$). Different doses of RUT did not make a statistical difference on MDA and GSH levels.

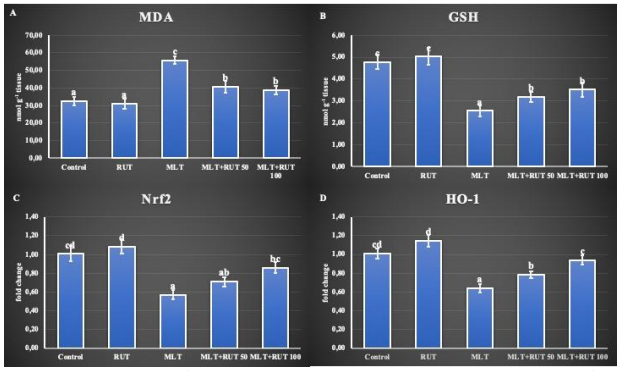

Figure 1. Status of oxidative stress markers in lung tissue after malathion and rutin treatments. (RUT: Rutin, MLT: Malathion, MDA: Malondialdehyde, GSH: Glutathione, Nrf2: Nuclear factor erythroid 2-related factor 2, HO-1: heme oxygenase 1)

3.2. mRNA Transcript Levels of Metalloproteinases in Lung Tissue

When the status of metalloproteinases in lung tissue was evaluated, it was determined that matrix metalloproteinase-2 (MMP2) and matrix metalloproteinase-9 (MMP9) expressions increased after MLT administration. It was observed that MMP expressions were down-regulated in the lung tissues of rats given RUT ($p<0.05$). In addition, another finding was that there was no difference in MMP2 or MMP9 expressions between MLT+RUT50 and MLT+RUT100 groups. The results are summarized in Figure 2.

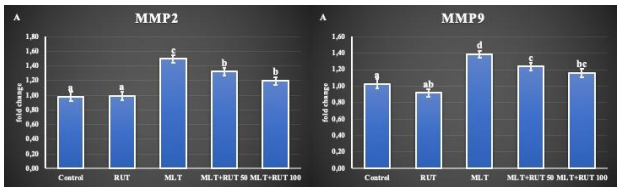

Figure 2. Status of metalloproteinases in lung tissue after malathion and its rutin treatments. (RUT: Rutin, MLT: Malathion, MMP2: matrix metalloproteinase-2, MMP9: matrix metalloproteinase-9)

3.3. Apoptotic State in Lung Tissue

After MLT and RUT treatments, mRNA transcript levels of Caspase-3 gene were analyzed by RT-PCR method to detect apoptotic state in lung tissue. According to the results presented in Figure 3, it was observed that MLT could trigger caspase-3 expression and cause apoptosis in lung tissue. On the other hand, it was determined that Caspase-3 expression was significantly suppressed after RUT administration and this was dose-dependent ($p<0.05$).

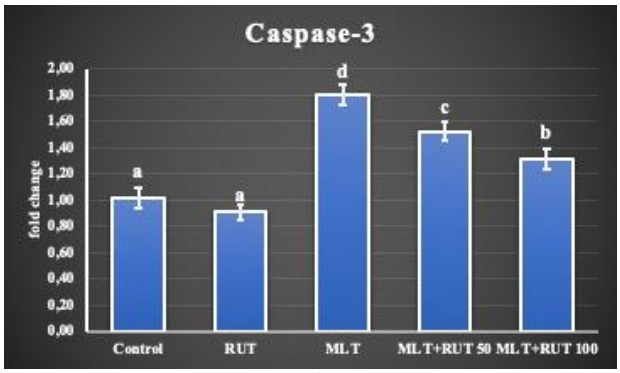

Figure 3. Apoptosis in lung tissue after malathion and rutin treatments. (RUT: Rutin, MLT: Malathion)

4. DISCUSSION AND CONCLUSION

Organophosphate pesticides are at the forefront of toxic substances, and many studies have shown that these compounds are among environmental pollutants that threaten human health. OPs are absorbed into the body through the respiratory system, gastrointestinal tract, and skin. OPs cause respiratory and neuromuscular conduction disorders mainly by inhibiting AChE in the nervous system [19]. MLT has taken its place among these toxic compounds as one of the environmental pollutants that threaten human health. Although many studies have been done against MLT toxicity before, an effective treatment method has not been developed yet [6, 20]. In the present study, the effects of RUT against the lung toxicity of MLT were evaluated, and the data obtained showed that RUT was a promising compound against this disease.

It has been previously reported that oxidative stress, which develops due to excessive increase in reactive oxygen species, is the basic mechanism of many toxic compounds [21-25]. There is evidence of increased oxidative stress in chronic toxicity of several OP compounds, including MLT [19, 26, 27]. However, information on the role of oxidative stress in subacute and chronic OP toxicity remains insufficient. OP is thought to bind directly to proteins and phosphorylate them, creating a pro-oxidative state [28]. In the present study, it was observed that the levels of GSH, a tripeptide antioxidant [29, 30], were significantly reduced in lung tissue after MLT treatment. Moreover, it was observed that decreased GSH levels probably caused a further increase in ROS levels and that these aggressive molecules damaged the membranes of lung tissue cells, causing lipid peroxidation, thereby increasing MDA levels. Previous studies have reported that different flavonoids applied against various pesticide groups alleviate oxidative stress and alleviate the toxic effects of these compounds [31, 32]. In our study, it was determined that RUT treatment suppressed oxidative stress by providing the regeneration of GSH, whose levels were significantly reduced by MLT, and by preventing lipid peroxidation.

Nrf2 is one of the transcription factors that protect cells against oxidative stress [33, 34]. Nrf2 regulates a wide range of genes such as superoxide dismutase, oxidoreductases and heme oxygenases [35, 36]. In a previous study, it was reported that deltamethrin suppressed Nrf2 as well as Cu/Zn SOD, GSH-Px, GST and CAT expressions in the liver and spleen tissue of *Channa argus*, however, significant increases in the mRNA transcript levels of these markers occurred after curcumin treatment [37]. Another study displayed that activating the Nrf2/HO-1 pathway exhibits nephroprotective effects in deltamethrin-induced nephrotoxicity [38]. Similarly, in the current study, it was determined that oxidative stress exacerbated due to the disruption of the Nrf2/HO-1 pathway after MLT treatment, but RUT treatment could activate this pathway and protect the lung tissue from MLT-induced oxidative stress. It is thought that the possible reason for this is the reduction of oxidative stress due to the ROS scavenging property of RUT and thus reducing the suppressive effect of oxidative stress on Nrf2.

Under normal conditions, the pulmonary extracellular matrix (ECM) determines the tissue architecture necessary for lung function. However, defects in the production of ECM are found in the fibrotic response [39]. MMPs are proteolytic enzymes known to be involved in cell migration and tissue remodeling [40]. Among MMPs, MMP2 and MMP9 contribute to tissue remodeling by playing a role in the degradation of collagen and elastase in lung tissue [41]. These endopeptidases have been reported to play an important role in various lung diseases [42]. Unfortunately, little is known about the effects of pesticides on MMPs in lung tissue. Several studies have reported that paraquat may contribute to the development and progression of fibrosis by increasing MMP9 expression [43, 44]. In the present study, it was observed that MLT contributed to pulmonary toxicity by triggering MMP expressions in the lung tissues of rats. On the other hand, it was determined that MMP2 and MMP9 expressions were downregulated with RUT treatment.

Apoptosis induced by factors such as reactive oxygen species is a normal cell death process [45, 46]. ROS production can cause cell damage due to increased membrane permeability and impaired mitochondrial function, resulting in apoptotic cell death [20]. Previous studies have shown that activation of the Nrf2/HO-1 signaling pathway plays a crucial role in inhibiting apoptosis by enhancing oxidative defense [45, 47]. Caspase-3 has an important role in the apoptotic pathway and is widely used as an apoptotic marker in many studies [48-51]. It has been reported that MLT treatment

increases apoptotic proteins in the brain such as Bax, Bak and caspase-3 [52, 53]. In the present study, it was observed that MLT could trigger the apoptotic process by up-regulating caspase-3 expression in lung tissue. On the other hand, it was determined that RUT treatment suppressed caspase-3 expression in anti-parallel with Nrf-2/HO-1 pathway. This supports the relationship between the lung toxicity of MLT and oxidative stress.

As a result, it was observed that oxidative stress and apoptosis were triggered in the lung tissues of rats given MLT, MMPs were activated and significant damage to the tissue could occur as a result. However, it has been determined that RUT treatment can alleviate MLT-induced pulmonary damage through suppression of oxidative stress, apoptosis and MMPs.

Acknowledgement

This study was carried out with the project numbered TSA-2021-8786 supported by Atatürk University Scientific Research Projects Coordination Unit.

REFERENCES

[1] Gupta VK, Siddiqi NJ, Ojha AK, Sharma B. Hepatoprotective effect of Aloe vera against cartap- and malathion-induced toxicity in Wistar rats. J Cell Physiol. 2019;234(10):18329-43.

[2] Pandit AA, Gandham RK, Mukhopadhyay CS, Verma R, Sethi R. Transcriptome analysis reveals the role of the PCP pathway in fipronil and endotoxin-induced lung damage. Respir res. 2019;20(1):1-16.

[3] Hassani S, Sepand M, Jafari A, Jaafari J, Rezaee R, Zeinali M, et al. Protective effects of curcumin and vitamin E against chlorpyrifos-induced lung oxidative damage. Hum Exp Toxicol. 2015;34(6):668-76.

[4] Jokanović M. Medical treatment of acute poisoning with organophosphorus and carbamate pesticides. Toxicol lett. 2009;190(2):107-15.

[5] Moore PD, Patlolla AK, Tchounwou PB. Cytogenetic evaluation of malathion-induced toxicity in Sprague-Dawley rats. MRGTEM. 2011;725(1-2):78-82.

[6] Abdel-Daim MM, Abushouk AI, Bungău SG, Bin-Jumah M, El-Kott AF, Shati AA, et al. Protective effects of thymoquinone and diallyl sulphide against malathion-induced toxicity in rats. Environ Sci Pollut Res. 2020;27(10):10228-35.

[7] Geng X, Shao H, Zhang Z, Ng JC, Peng C. Malathion-induced testicular toxicity is associated with spermatogenic apoptosis and alterations in testicular enzymes and hormone levels in male Wistar rats. Environ Toxicol Pharmacol. 2015;39(2):659-67.

[8] Uysal M, Karaman S. In vivo effects of intravenous lipid emulsion on lung tissue in an experimental model of acute malathion intoxication. Toxicol Ind. 2018;34(2):110-8.

[9] Jahan S, Munawar A, Razak S, Anam S, Ain QU, Ullah H, et al. Ameliorative effects of rutin against cisplatin-induced reproductive toxicity in male rats. BMC urology. 2018;18(1):1-11.

[10] Nafees S, Rashid S, Ali N, Hasan SK, Sultana S. Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: role of NFκB/MAPK pathway. Chem Biol Interact. 2015;231:98-107.

[11] Zargar S, Wani TA, Alamro AA, Ganaie MA. Amelioration of thioacetamide-induced liver toxicity in Wistar rats by rutin. Int J Immunopathol Pharmacol. 2017;30(3):207-14.

[12] Yeh C-H, Yang J-J, Yang M-L, Li Y-C, Kuan Y-H. Rutin decreases lipopolysaccharide-induced acute lung injury via inhibition of oxidative stress and the MAPK–NF-κB pathway. Free Radic Biol Med. 2014;69:249-57.

[13] Kandemir FM, Caglayan C, Aksu EH, Yildirim S, Kucukler S, Gur C, et al. Protective effect of rutin on mercuric chloride-induced reproductive damage in male rats. Andrologia. 2020;52(3):e13524.

[14] 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;56:60-8.

[15] Akbel E, Arslan-Acaroz D, Demirel HH, Kucukkurt I, Ince S. The subchronic exposure to malathion, an organophosphate pesticide, causes lipid peroxidation, oxidative stress, and tissue damage in rats: the protective role of resveratrol. Toxicol res. 2018;7(3):503-12.

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

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

[18] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 2001;25(4):402-8.

[19] Farkhondeh T, Mehrpour O, Forouzanfar F, Roshanravan B, Samarghandian S. Oxidative stress and mitochondrial dysfunction in organophosphate pesticide-induced neurotoxicity and its amelioration: a review. Environ Sci Pollut Res. 2020;27(20):24799-814.

[20] Ranjbar A, Ghahremani MH, Sharifzadeh M, Golestani A, Ghazi-Khansari M, Baeeri M, et al. Protection by pentoxifylline of malathion-induced toxic stress and mitochondrial damage in rat brain. Hum Exp Toxicol. 2010;29(10):851-64.

[21] Caglayan C, Temel Y, Kandemir FM, Yildirim S, Kucukler S. Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. Environ Sci Pollut Res. 2018;25(21):20968-84.

[22] Gur C, Kandemir O, Kandemir FM. Investigation of the effects of hesperidin administration on abamectin-induced testicular toxicity in rats through oxidative stress, endoplasmic reticulum stress, inflammation, apoptosis, autophagy, and JAK2/STAT3 pathways. Environ Toxicol. 2022;37(3):401-12.

[23] Gur C, Kandemir FM, Darendelioglu E, Caglayan C, Kucukler S, Kandemir O, et al. Morin protects against acrylamide-induced neurotoxicity in rats: an investigation into different signal pathways. Environ Sci Pollut Res. 2021;28(36):49808-19.

[24] Kucukler S, Benzer F, Yildirim S, Gur C, Kandemir FM, Bengu AS, et al. Protective effects of chrysin against oxidative stress and inflammation induced by lead acetate in rat kidneys: a biochemical and histopathological approach. Biol Trace Elem Res. 2021;199(4):1501-14.

[25] Yıldız MO, Çelik H, Caglayan C, Kandemir FM, Gür C, Bayav İ, et al. Neuromodulatory effects of hesperidin against sodium fluoride-induced neurotoxicity in rats: Involvement of neuroinflammation, endoplasmic reticulum stress, apoptosis and autophagy. NeuroToxicology. 2022;90:197-204.

[26] Coban FK, Ince S, Kucukkurt I, Demirel HH, Hazman O. Boron attenuates malathion-induced oxidative stress and acetylcholinesterase inhibition in rats. Drug Chem Toxicol. 2015;38(4):391-9.

[27] Ince S, Arslan-Acaroz D, Demirel HH, Varol N, Ozyurek HA, Zemheri F, et al. Taurine alleviates malathion induced lipid peroxidation, oxidative stress, and proinflammatory cytokine gene expressions in rats. Biomed Pharmacother. 2017;96:263-8.

[28] Vanova N, Pejchal J, Herman D, Dlabkova A, Jun D. Oxidative stress in organophosphate poisoning: role of standard antidotal therapy. J Appl Toxicol. 2018;38(8):1058-70.

[29] Gür C, Genç A, Kandemir Ö. Ratlarda Deltametrin'in Neden Olduğu Dalak Toksisitesine Karşı Rutin'in Potansiyel Koruyucu Etkilerinin Oksidatif Stres, Apoptoz ve İnflamasyon Belirteçleri Üzerinden Araştırılması. Atatürk University J Vet Sci. 17(1):6-10.

[30] Caglayan C, Kandemir FM, Darendelioğlu E, Küçükler S, Ayna A. Hesperidin protects liver and kidney against sodium fluoride-induced toxicity through anti-apoptotic and anti-autophagic mechanisms. Life Sci. 2021;281:119730.

[31] Küçükler S, Çomaklı S, Özdemir S, Çağlayan C, Kandemir FM. Hesperidin protects against the chlorpyrifos-induced chronic hepato-renal toxicity in rats associated with oxidative stress, inflammation, apoptosis, autophagy, and up-regulation of PARP-1/VEGF. Environ Toxicol. 2021;36(8):1600-17.

[32] Anwar M, Muhammad F, Akhtar B, Saleemi MK. Nephroprotective effects of curcumin loaded chitosan nanoparticles in cypermethrin induced renal toxicity in rabbits. Environ Sci Pollut Res. 2020;27(13):14771-9.

[33] Yardim A, Gur C, Comakli S, Ozdemir S, Kucukler S, Celik H, et al. Investigation of the effects of berberine on bortezomib-induced sciatic nerve and spinal cord damage in rats through pathways involved in oxidative stress and neuro-inflammation. Neurotoxicology. 2022;89:127-39.

[34] Yardim A, Kandemir FM, Ozdemir S, Kucukler S, Comakli S, Gur C, et al. Quercetin provides protection against the peripheral nerve damage caused by vincristine in rats by suppressing caspase 3, NF-κB, ATF-6 pathways and activating Nrf2, Akt pathways. Neurotoxicology. 2020;81:137-46.

[35] Hussein RM, Mohamed WR, Omar HA. A neuroprotective role of kaempferol against chlorpyrifos-induced oxidative stress and memory deficits in rats via GSK3β-Nrf2 signaling pathway. Pestic Biochem Physiol. 2018;152:29-37.

[36] Yardim A, Gur C, Comakli S, Ozdemir S, Kucukler S, Celik H, et al. Investigation of the effects of berberine on bortezomib-induced sciatic nerve and spinal cord damage in rats through pathways involved in oxidative stress and neuro-inflammation. Neurotoxicology. 2022;89:127-39.

[37] Kong Y, Li M, Guo G, Yu L, Sun L, Yin Z, et al. Effects of dietary curcumin inhibit deltamethrin-induced oxidative stress, inflammation and cell apoptosis in Channa argus via Nrf2 and NF-κB signaling pathways. Aquaculture. 2021;540:736744.

[38] Shi W, Zhang D, Wang L, Sreeharsha N, Ning Y. Curcumin synergistically potentiates the protective effect of sitagliptin against chronic deltamethrin nephrotoxicity in rats: impact on pro-inflammatory cytokines and Nrf2/Ho-1 pathway. JBMT. 2019;33(10):e22386.

[39] Subbiah R, Tiwari RR. The herbicide paraquat-induced molecular mechanisms in the development of acute lung injury and lung fibrosis. Crit Rev Toxicol. 2021;51(1):36-64.

[40] Zhang W, Fievez L, Cheu E, Bureau F, Rong W, Zhang F, et al. Anti-inflammatory effects of formoterol and ipratropium bromide against acute cadmium-induced pulmonary inflammation in rats. Eur J Pharmacol. 2010;628(1-3):171-8.

[41] 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):1201-11.

[42] Zhang F, Hu L, Wu Y-x, Fan L, Liu W-t, Wang J, et al. Doxycycline alleviates paraquat-induced acute lung injury by inhibiting neutrophil-derived matrix metalloproteinase 9. Int Immunopharmacol. 2019;72:243-51.

[43] Liu M-w, Liu R, Wu H-y, Li Y-y, Su M-x, Dong M-n, et al. Radix puerariae extracts ameliorate paraquat-induced pulmonary fibrosis by attenuating follistatin-like 1 and nuclear factor erythroid 2p45-related factor-2 signalling pathways through downregulation of miRNA-21 expression. BMC Complement Altern Med. 2015;16(1):1-15.

[44] Yao R, Cao Y, He Y-r, Lau WB, Zeng Z, Liang Z-a. Adiponectin attenuates lung fibroblasts activation and pulmonary fibrosis induced by paraquat. PLoS One. 2015;10(5):e0125169.
[45] Yang X, Fang Y, Hou J, Wang X, Li J, Li S, et al. The heart as a target for deltamethrin toxicity: Inhibition of Nrf2/HO-1 pathway induces oxidative stress and results in inflammation and apoptosis. Chemosphere. 2022;300:134479.
[46] Varışlı B, Darendelioğlu E, Caglayan C, Kandemir FM, Ayna A, Genç A, et al. Hesperidin Attenuates Oxidative Stress, Inflammation, Apoptosis, and Cardiac Dysfunction in Sodium Fluoride-Induced Cardiotoxicity in Rats. Cardiovasc Toxicol. 2022;23:1-9.
[47] Hou W, Zhu X, Liu J, Map J. Inhibition of miR-153 ameliorates ischemia/reperfusion-induced cardiomyocytes apoptosis by regulating Nrf2/HO-1 signaling in rats. Biomed Eng Online. 2020;19(1):1-14.
[48] 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.
[49] 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):486-98.
[50] Kandemir FM, Kucukler S, Caglayan C, Gur C, Batil AA, Gülçin İ. Therapeutic effects of silymarin and naringin on methotrexate-induced nephrotoxicity in rats: Biochemical evaluation of anti-inflammatory, antiapoptotic, and antiautophagic properties. J Food Biochem. 2017;41(5):e12398.
[51] Caglayan C, Kandemir FM, Ayna A, Gür C, Küçükler S, Darendelioğlu E. Neuroprotective effects of 18β-glycyrrhetinic acid against bisphenol A-induced neurotoxicity in rats: involvement of neuronal apoptosis, endoplasmic reticulum stress and JAK1/STAT1 signaling pathway. Metab Brain Dis. 2022;37:1931-40.
[52] Salama OA, Attia MM, Abdelrazek MA. Modulatory effects of swimming exercise against malathion induced neurotoxicity in male and female rats. Pestic Biochem Physiol. 2019;157:13-8.
[53] Dos Santos AA, Naime AA, de Oliveira J, Colle D, Dos Santos DB, Hort MA, et al. Long-term and low-dose malathion exposure causes cognitive impairment in adult mice: evidence of hippocampal mitochondrial dysfunction, astrogliosis and apoptotic events. Arch toxicol. 2016;90(3):647-60.