Cilt 12, Sayı 2, Sayfa 132-137, 2023 Volume 12, Issue 2, Page 132-137, 2023
Araştırma Makalesi https://doi.org/10.46810/tdfd.1294861 Research Article
BİNGÖL ÜNİVERSİTESİ
Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Carvacrol Ameliorates Sodium Arsenite-Induced Intestinal Toxicity

Mustafa İLERİTÜRK[1*], Özge KANDEMİR[2]

[1] Ataturk University, Horasan Vocational College, Department of Animal Science, Erzurum, Türkiye
[2] Aksaray University, Aksaray Technical Sciences Vocational School, Aksaray, Türkiye
Mustafa İLERİTÜRK ORCID No: 0000-0002-4581-4492
Özge KANDEMİR ORCID No: 0000-0001-8884-4168

**Corresponding author: m.ileriturk@atauni.edu.tr*

(Received: 10.05.2023, Accepted: 15.06.2023, Online Publication: 22.06.2023)

Keywords
Apoptosis, Carvacrol, Inflammation, Intestinal toxicity, Oxidative stress, Sodium arsenite

Abstract: Arsenic is a very dangerous metal that is widely distributed in the environment as a result of anthropogenic and natural processes. On the other hand, it is thought that treatment with natural flavonoids may protect against arsenic toxicity. Carvacrol (CRV) is a naturally occurring phenolic compound with anti-inflammatory, anti-apoptotic, and antioxidant properties. This study aims to investigate the protective effects of CRV on sodium arsenite (SA)-induced intestinal toxicity. For this purpose, rats were randomly divided into five groups and administered SA and CRV orally for 14 days. The data indicate that when SA is administered, lipid peroxidation increase and antioxidant enzyme activities decrease. The administration of CRV ameliorated this impairment in the antioxidant defense system. Examining the expression levels of NF-κB and IL-1β revealed that inflammation increased with SA application but decreased with CRV administration. Moreover, the expression levels of Caspase-3 and Apaf1 increased in rats treated with SA, whereas the severity of apoptosis decreased when CRV was administered. In light of these findings, it is possible to state that CRV protects tissues from damage by presenting antioxidant, anti-inflammatory, and anti-apoptotic effects in SA-induced intestinal toxicity.

Carvacrol, Sodyum Arsenit Kaynaklı İnce Bağırsak Toksisitesini İyileştirmektedir

Anahtar Kelimeler
Apoptoz, Carvacrol, İnce bağırsak toksisitesi, İnflamasyon, Oksidatif stres, Sodyum arsenit

Öz: Arsenik, doğal ve antropojenik faaliyetler yoluyla çevrede bol miktarda bulunan son derece tehlikeli bir metaldir. Bununla beraber doğal flavonoidlerle tedavinin arsenik toksisitesine karşı koruyucu etkisi olabileceği öngörülmektedir. Carvacrol (CRV) antioksidan, anti-inflamatuvar ve antiapoptotik özellikleri bilinen doğal bir fenolik bileşiktir. Bu çalışmanın amacı sodyum arsenit (SA) ile oluşturulan ince bağırsak toksisitesinde CRV'nin koruyucu etkilerinin araştırılmasıdır. Bu amaçla ratlar rastgele 5 gruba ayrıldı ve 14 gün boyunca oral olarak SA ve CRV uygulaması gerçekleştirildi. Veriler SA uygulaması ile lipid peroksidasyonunun şekillendiğini antioksidan enzim aktivitelerinin ise azaldığını göstermektedir. Antioksidan savunma durumundaki bu bozukluk CRV uygulaması ile azaldı. NF-κB ve IL-1β ekspresyon düzelerine bakıldığına SA uygulaması ile inflamasyonun şiddetlendiği CRV uygulaması ile bu şiddetin azaldığı tespit edildi. Buna ek olarak yine SA uygulanan ratlarda artan Caspase-3 ve Apaf1 ekspresyon düzeylerinin CRV uygulaması ile düştüğü ve apoptozun şiddetinin azaldığı belirlendi. Tüm bu veriler ışığında SA kaynaklı ince bağırsak toksisitesinde CRV'nin antioksidan, anti-inflamatuvar ve antiapoptotik etki göstererek dokuları hasarda koruduğu söylenebilir.

1. INTRODUCTION

The health effects of arsenic are becoming more serious worldwide [1]. The main source of arsenic exposure is drinking water that has more arsenic than is allowed. Drinking water may get contaminated with arsenic due to natural deposits, industrial processes, and excessive use of agricultural pesticides and rodenticides [2]. Multiple organs, including intestine, are affected by arsenic [3]. Arsenic is also a well-known cancer-causing substance that results in a variety of malignant tumors in people [4]. The most extensively researched and recognized mechanism for arsenic poisoning is oxidative stress, despite the fact that it has not yet been fully understood.

Lipid peroxidation and reactive oxygen species (ROS) are both increased by arsenic, which leads to organ damage [5]. In addition, arsenic depletes antioxidants that are protective, including glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Therefore, by reducing the impact of reactive oxygen metabolites on cellular components, increasing cellular antioxidant capacity may be useful in limiting the toxicity of arsenic.

Protein inactivation and an increase in ROS are caused by arsenic binding to the sulfhydryl groups of proteins and glutathione [6, 7]. Additionally, it has been shown that increased ROS may damage DNA and trigger pathways that result in cell death [8]. Due to this, antioxidant molecules are thought to be a potential therapy for arsenic poisoning, and interest in using naturally occurring phytochemicals is growing daily .

Carvacrol (CRV), also known as 5-isopropyl-2-methylphenol, is a phenolic monoterpene present in the essential oils of many Lamiaceae plants [9, 10]. This substance was shown to have antioxidant, anti-inflammatory, anticancer, antispasmodic, and chemoprotective properties in earlier in vitro and in vivo research [11, 12]. According to studies, CRV not only improves endogenous antioxidant levels but also defends against inflammation, which is the underlying cause of many diseases, by controlling the expression of genes that are essential for inflammation [13].

The purpose of this study was to determine whether CRV has protective effects against sodium arsenite-induced intestinal toxicity.

2. MATERIAL AND METHOD

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

35 male Sprague-Dawley rats weighing between 220 and 250 g and aged 10 to 12 weeks were used in the experiment. Ataturk University Medical Experimental Application and Research Center provided the animals. They had free access to water and standard laboratory pellet feeds. They were kept in an environment with a temperature of 24±1 °C, humidity of 45±5 %, and a light/dark cycle of 12 hours. Before administering the compounds, the animals were observed for one week to corroborate that they had adapted to the environment. All animal investigations were conducted at the Medical Experimental Research Center of Ataturk University. The Ataturk University Animal Experiments Local Ethics Committee approved the investigation (Protocol Number: 2022-11-226) with ethics committee approval.

2.2. Experimental Procedure

Rats were randomly divided into 5 groups, with 7 animals in each group.

- *Control Group*: For 14 days, physiological saline was administered orally.
- *CRV Group*: For 14 days, 50 mg/kg CRV was orally administered to rats.
- *SA Group*: The rats received 10 mg/kg sodium arsenite (SA) orally for 14 days.
- *SA + CRV 25 Group*: The rats received 10 mg/kg SA for 14 days, followed by 25 mg/kg CRV administered 30 minutes later.
- *SA + CRV 50 Group*: The rats received 10 mg/kg SA for 14 days, followed by 50 mg/kg CRV administered 30 minutes later.

24 hours after the last sodium arsenide administration, animals were decapitated under mild sevoflurane anesthesia. Intestine tissue was stored at −80 °C for biochemical and Real-Time PCR studies.

2.3. Preparation of Tissue Homogenates

To generate the intestine tissue homogenate, the tissues were diluted 1:20 with phosphate-buffered saline (PBS; pH 7.4). With a tissue lysate device (TissueLyser II, Qiagen), the resulting mixture was quickly homogenized. 30 minutes at +4 °C and 3000 rpm were spent centrifuging the homogenate. Analysis was conducted using the supernatant.

2.4. Determination of Oxidative Stress Status

Using the method established by Placer et al.[14], the level of lipid peroxidation was determined by quantifying the amount of malondialdehyde (MDA). SOD activity was determined using a method developed by Sun et al.[15]. The catalase (CAT) activity was measured using a method established by Aebi [16], and the data were expressed as catal/g protein. GPx activity was assessed using a technique created by Lawrence and Burk [17]. GSH levels were determined using the method described by Sedlak and Lindsay [18]. The Lowry et al.[19] technique was used to analyze total protein, with bovine serum albumin serving as the reference.

2.5. Real -Time PCR Analysis

After collecting intestinal tissues from animals treated with sodium arsenide and carvacrol, the expression levels of the genes Nrf2, HO-1, NF-κB, IL-1β, Caspase-3, and Apaf-1 were examined using the RT-PCR technique. In accordance with the manufacturer's instructions, tissues were treated with QIAzol Lysis Reagent (79306; Qiagen) to obtain total RNAs. Concentrations of RNAs were measured in NanoDrop (BioTek Epoch). The obtained RNAs were then transformed into double-stranded cDNAs using the iScript cDNA Synthesis Kit (Bio-Rad). This procedure was carried out in accordance with the manufacturer's instructions, and the following reaction conditions were established:

1. Priming: 5 minutes at 25 °C
2. Reverse transcription: 20 minutes at 46 °C
3. RT inactivation: 1 minute at 95 °C

In the final phase of the analysis, cDNAs, primers of the pertinent genes, and the mixture prepared with iTaq

Universal SYBR Green Supermix (BIORAD) were reacted in the Rotor-Gene Q (Qiagen) device using the time and temperature cycles indicated by the manufacturer. Following the completion of the cycles, the CT values provided by the device were normalized according to β-Actin. This process was performed according to the $2^{-\Delta\Delta CT}$ method [20]. Primer sequences have been presented in Table 1.

Table 1. Primer sequences

Gene	Sequences (5'-3')	Length (bp)
NF-κB	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	106
IL-1β	F: ATGGCAACTGTCCCTGAACT R: AGTGACACTGCCTTCCTGAA	197
Apaf-1	F: ACCTGAGGTGTCAGGACC R: CCGTCGAGCATGAGCCAA	192
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270
Nrf2	F: TTTGTAGATGACCATGAGTCGC R: TCCTGCCAAACTTGCTCCAT	161
HO-1	F: ATGTCCCAGGATTTGTCCGA R: ATGGTACAAGGAGGCCATCA	144
β-Actin	F: CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	360

2.6. Statistical Analysis

One-way analysis of variance (One-way ANOVA) was used to perform a statistical analysis of the biochemical and Real-Time PCR data. Tukey's multiple comparison test was employed to determine whether there was a significant difference between the groups. Results were shown as mean ± standard deviation. Statistical significance was defined as a p value <0.05.

3. RESULTS

3.1. Effect of CRV on Lipid Peroxidation in Sa-Induced Intestine Toxicity

Table 2 shows the effects of CRV against SA-induced lipid peroxidation in intestine tissue. Compared with the control group, SA increased MDA levels, an essential biomarker of lipid peroxidation, by approximately 1.7-fold. The levels of SA-induced lipid peroxidation reduced with CRV and approached those of the control group. The therapeutic efficacy of the CRV doses of 25 mg/kg and 50 mg/kg was also statistically significantly different ($p<0.01$).

3.2. Effect of CRV on Enzymatic and Non-Enzymatic Antioxidant Markers in SA-Induced Intestine Toxicity

In the study, it was determined that SA reduced the activity of antioxidant enzymes relative to the control group ($p<0.001$). In addition, GSH stores, an essential antioxidant in the body, were found to decrease by approximately 37% in response to SA treatment. This indicates that SA causes oxidative stress in intestine tissue. As contrast to the SA group, the CRV-treated groups showed higher GSH levels and SOD, CAT, and GPx activities that were closer to those of the control group ($p<0.001$). In addition, there was a statistically significant difference between the 25 mg/kg and 50 mg/kg doses of CRV for CAT and GPx ($p<0.001$, $p<0.01$, respectively). Table 2 presents the data for enzymatic and non-enzymatic indicators in intestine tissue.

3.3. Effect of CRV on Nrf2 and HO-1 in SA-Induced Intestine Toxicity

In addition to biochemical analyses, the mRNA expression levels of Nrf-2 and HO-1 were measured to further clarify the oxidative stress state. According to the obtained results, the expression levels of Nrf-2 and HO-1 in rats administered SA were lower than those in the control group ($p<0.001$). It was found that CRV co-administration reversed this expression decrease and regulated the antioxidant defense mechanism. There was a significant difference between low-dose and high-dose CRV administration ($p<0.05$ and $p<0.001$, respectively). Figue 1 shows the mRNA trancription levels on Nrf2 and HO-1.

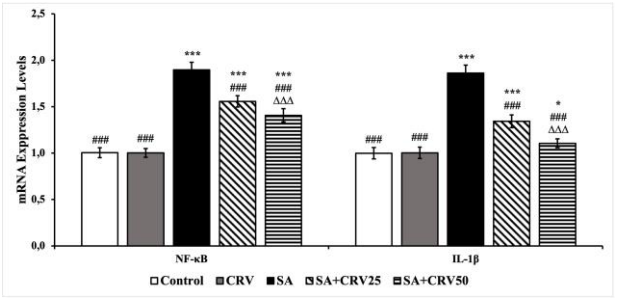

Figure 1. The mRNA transcript level of Nrf2 and HO-1 in the intestine of rats. All data were expressed as mean ± SD. Statistical significance (Control vs others: *p< 0.05, **p < 0.01, ***p < 0.001, SA vs others: #p< 0.05, ##p < 0.01, ###p < 0.001, SA + CRV 25 vs SA + CRV 50: Δp< 0.05, ΔΔp < 0.01, ΔΔΔp < 0.001) was analyzed using One Way ANOVA.

Table 2. Effect of CRV on oxidative stress biomarkers in SA-induced intestinal toxicity

	Control	CRV	SA	SA+CRV 25	SA+CRV 50
MDA (nmol/g tissue)	$75,3 \pm 5,6^{\#\#\#}$	$73,2 \pm 4,3^{\#\#\#}$	$126,1 \pm 8,7^{***}$	$110,0 \pm 6,6^{***}$	$83,8 \pm 6,3^{\#\#\#/\Delta\Delta}$
GSH (nmol/g tissue)	$6,2 \pm 0,5^{\#\#\#}$	$7,0 \pm 0,5^{\#\#\#}$	$3,9 \pm 0,4^{***}$	$4,4 \pm 0,3^{**}$	$5,3 \pm 0,5^{\#}$
CAT (katal/g protein)	$25,1 \pm 1,6^{\#\#\#}$	$23,7 \pm 1,4^{\#\#\#}$	$12,9 \pm 0,9^{***}$	$15,2 \pm 1,0^{***}$	$21,2 \pm 1,2^{**/\#\#\#/\Delta\Delta\Delta}$
SOD (U/g tissue)	$11,0 \pm 0,9^{\#\#\#}$	$11,3 \pm 0,8^{\#\#\#}$	$5,7 \pm 0,5^{***}$	$7,4 \pm 0,7^{***}$	$9,2 \pm 0,7^{\#\#\#}$
GPx (U/g tissue)	$28,9 \pm 1,6^{\#\#\#}$	$27,6 \pm 1,7^{\#\#\#}$	$13,4 \pm 1,0^{***}$	$18,5 \pm 1,3^{\#\#/***}$	$24,5 \pm 1,2^{*/\#\#\#/\Delta\Delta}$

Statistical significance (Control vs others: *p< 0.05, **p < 0.01, ***p < 0.001, SA vs others: #p< 0.05, ##p < 0.01, ###p < 0.001, SA + CRV 25 vs SA + CRV 50: Δp< 0.05, ΔΔp < 0.01, ΔΔΔp < 0.001) was analyzed using One Way ANOVA.

3.4. Effect of CRV on Inflammation in SA-Induced Intestine Toxicity

Real-time PCR was used to determine the NF-κB and IL-1β mRNA transcript levels in intestine tissue to determine the inflammation. Analysis of mRNA transcripts revealed that NF-κB and IL-1β increased considerably in the SA group compared to the control group ($p<0.001$ for both). The levels of NF-κB and IL-1β were decreased in the CRV-treated group compared to the SA-treated group ($p<0.001$ for both dosages). Figure 2 shows the mRNA transcript levels of NF-κB and IL-1β.

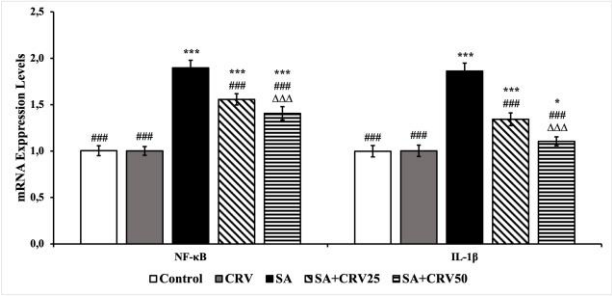

Figure 2. The mRNA transcript level of NF-κB and IL-1β in the intestine of rats. All data were expressed as mean ± SD. Statistical significance (Control vs others: *p< 0.05, **p < 0.01, ***p < 0.001, SA vs others: #p< 0.05, ##p < 0.01, ###p < 0.001, SA + CRV 25 vs SA + CRV 50: Δp< 0.05, ΔΔp < 0.01, ΔΔΔp < 0.001) was analyzed using One Way ANOVA.

3.5. Effect of CRV on Apoptosis in SA-Induced Intestine Toxicity

The degree of apoptosis in intestine tissue was determined by analyzing mRNA transcript levels of Caspase-3 and Apaf1. According to the findings of mRNA transcripts, Caspase-3 and Apaf1 levels in the SA group were 2.21 and 2.25 times higher, respectively, than in the control group (Figure 3). With CRV co-administration, Caspase-3 and Apaf1 transcript levels decreased compared to SA alone ($p<0.001$). Evaluating the difference between the two groups revealed that the 50 mg/kg dosage was more effective than the 25 mg/kg dose ($p<0.001$).

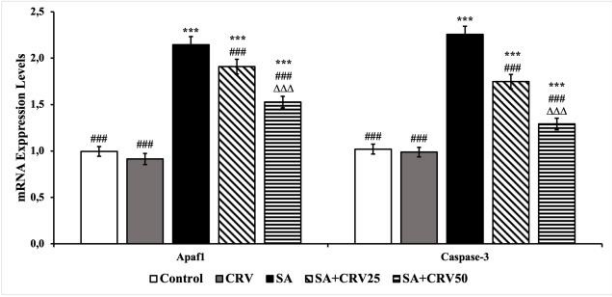

Figure 3. The mRNA transcript level of Caspase-3 and Apaf1 in the intestine of rats. All data were expressed as mean ± SD. Statistical significance (Control vs others: *p< 0.05, **p < 0.01, ***p < 0.001, SA vs others: #p< 0.05, ##p < 0.01, ###p < 0.001, SA + CRV 25 vs SA + CRV 50: Δp< 0.05, ΔΔp < 0.01, ΔΔΔp < 0.001) was analyzed using One Way ANOVA.

4. DISCUSSION AND CONCLUSION

Arsenite is prevalent in the environment and is harmful to virtually all body tissues; therefore, it is essential to focus on alternative therapies to mitigate and avoid its effects [21, 22]. Recent research on the efficacy of natural flavonoids against the toxicity of heavy metals suggests that these compounds may be useful in mitigating and avoiding the effects of arsenite. Arsenite exposure is damaging to tissues in a variety of ways, some of the most widely acknowledged of which include an increase in ROS that leads to oxidative stress. Because ROS contribute to the pathogenesis of arsenite toxicity, their elimination should be one of the protective targets [23, 24]. A natural herb, CRV contains anti-inflammatory and antioxidant properties [25]. However, it has not yet been investigated for its possible protective effect against arsenite-induced intestinal damage. This study investigated the anti-inflammatory, antioxidant, and antiapoptotic effects of CRV on the SA-induced intestinal toxicity of rats.

The initial line of defense against reactive oxygen species damage is provided by enzymes like SOD, CAT, and GPx. CAT and GPx break down hydrogen peroxide into water and molecular oxygen, whereas SOD catalyzes the transformation of superoxide radical into extremely dangerous hydrogen peroxide and normal molecular oxygen, which can cause persistent cell damage [26, 27]. Free radicals, peroxides, and heavy metals are just a few examples of the ROS that may harm cells, and GSH is a nonenzymatic antioxidant that protects against these effects. GSH can become oxidized (GSSG) when it binds free radicals, and the GSSG/GSH ratio is a significant indicator of oxidative stress [28, 29]. MDA is an indicator that membrane lipids have been damaged by oxidation, which is strongly linked to the amount of lipid peroxidation [30, 31]. It has been demonstrated that SA inhibits the activity of antioxidant enzymes and causes oxidative stress and lipid peroxidation [32]. This study investigated the protective and beneficial effects of CRV on SA-induced intestinal toxicity. It was found that SA increased oxidative stress and decreased the antioxidant system in intestinal tissue. On the other hand, it was discovered that CRV reduced intestinal injury by reducing the effectiveness of free radicals and activating the antioxidant system. It was discovered that SA caused oxidative damage by inducing an inverse relationship between SOD, CAT, and GPx enzyme activity and MDA concentrations in intestinal tissues. Alternatively, it was discovered that CRV inhibits the deterioration of the oxidant-antioxidant balance by scavenging free radicals, resulting in a decrease in MDA levels by restoring antioxidant enzyme activity and replenishing GSH reserves.

The transcription factor NF-κB plays a crucial role in the production of inflammatory cytokines and the onset of the immune response in a variety of tissues [33-35]. In a previous study, it was discovered that SA promotes inflammation in tissues via triggering NF-κB activation [36]. In the present study, it was shown that SA increased NF-κB transcript levels in intestinal tissues, leading to oxidative stress and tissue damage; CRV treatment given in combination with SA decreased NF-κB transcript levels and intestinal toxicity. The secretion of the proinflammatory mediator IL-1β is decreased by preventing the translocation of NF-κB to the nucleus. In

the present study, an increase in IL-1β transcript levels was found, and it was hypothesized that this was due to an increase in NF-κB gene expressions resulting from SA-induced ROS production.

Cell death occurs naturally via a process called apoptosis [37, 38]. However, tissue function becomes disrupted when it develops excessively [39]. Apoptotic factors Caspase-3 and Apaf1 play crucial roles in the apoptotic process and are commonly used biomarkers for determining apoptotic state. In our research, SA treatment increased Caspase-3 and Apaf1 expressions. On the other hand, it has been discovered that CRV may inhibit Caspase-3 and Apaf1 expressions, thereby reducing apoptosis. Similarly, a recent study showed that Caspase-3 expression increased significantly after CRV administration [6].

As a result, it was found that CRV had anti-oxidant, anti-inflammatory, and anti-apoptotic effects in intestinal toxicity induced by SA, decreasing ROS formation and healing tissue and organ damage. It was therefore determined that utilizing CRV as a supportive treatment in SA toxicity would be beneficial.

REFERENCES

[1] Nikravesh M, Mahdavinia M, Neisi N, Khorsandi L, Khodayar MJ. Citicoline ameliorates arsenic-induced hepatotoxicity and diabetes in mice by overexpression of VAMP2, PPAR-gamma, As3MT, and SIRT3. Pestic Biochem Physiol. 2023;192:105391.

[2] Anetor JJ, Wanibuchi H, Fukushima S. Arsenic exposure and its health effects and risk of cancer in developing countries: Micronutrients as host defence. Asian Pac J Cancer P. 2007;8(1):13-23.

[3] Liu RQ, Kong SQ, Shao YX, Cai DW, Bai B, Wei XG, et al. Chorover, Mechanisms and health implications of toxicity increment from arsenate-containing iron minerals through in vitro gastrointestinal digestion. Geoderma. 2023;432:116377.

[4] Brown KG, Ross GL. Arsenic, drinking water, and health: A position paper of the American Council on Science and Health. Regul Toxicol Pharm. 2002;36(2):162-174.

[5] Caglayan C, Demir Y, Kucukler S, Taslimi P, Kandemir FM, Gulcin I. The effects of hesperidin on sodium arsenite-induced different organ toxicity in rats on metabolic enzymes as antidiabetic and anticholinergics potentials: A biochemical approach. J Food Biochem. 2019;43(2): e12720.

[6] Akaras N, Gur C, Kucukler S, Kandemir FM. Zingerone reduces sodium arsenite-induced nephrotoxicity by regulating oxidative stress, inflammation, apoptosis and histopathological changes. Chem Biol Interact. 2023;374:110410.

[7] Kuzu M, Kandemir FM, Yildirim S, Caglayan C, Kucukler S. Attenuation of sodium arsenite-induced cardiotoxicity and neurotoxicity with the antioxidant, anti-inflammatory, and antiapoptotic effects of hesperidin. Environ Sci Pollut Res Int. 2021;28(9):10818-10831.

[8] Turk E, Kandemir FM, Yildirim S, Caglayan C, Kucukler S, Kuzu M. Protective Effect of Hesperidin on Sodium Arsenite-Induced Nephrotoxicity and Hepatotoxicity in Rats. Biol Trace Elem Res. 2019;189(1):95-108.

[9] 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. Molecular Biology Reports. 2022;49(2):1201-1211.

[10] Nouri A, Izak-Shirian F, Fanaei V, Dastan M, Abolfathi M, Moradi A, Khaledi M, Mirshekari-Jahangiri H. Carvacrol exerts nephroprotective effect in rat model of diclofenac-induced renal injury through regulation of oxidative stress and suppression of inflammatory response. Heliyon. 2021;7(11): e08358.

[11] Kandemir FM, Caglayan C, Darendelioglu E, Kucukler S, Izol E, Kandemir O. Modulatory effects of carvacrol against cadmium-induced hepatotoxicity and nephrotoxicity by molecular targeting regulation. Life Sciences. 2021;277:119610.

[12] 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: 1265-1276.

[13] Riaz M, Al Kury LT, Atzaz N, Alattar A, Alshaman R, Shah FA, et al. Carvacrol Alleviates Hyperuricemia-Induced Oxidative Stress and Inflammation by Modulating the NLRP3/NF-kappa B. Drug Des Dev Ther. 2022;16:1159-1170.

[14] Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem. 1966;16(2):359-364.

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

[16] Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-126.

[17] Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun. 1976;71(4):952-958.

[18] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205.

[19] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-275.

[20] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-408.

[21] Oyagbemi AA, Omobowale TO, Asenuga ER, Ochigbo GO, Adejumobi AO, Adedapo AA, et al. Sodium arsenite-induced cardiovascular and renal

dysfunction in rat via oxidative stress and protein kinase B (Akt/PKB) signaling pathway. Redox Report. 2017;22(6):467-477.
[22] Oyibo A, Gbadegesin MA, Odunola OA. Ethanol extract of Vitellaria paradoxa (Gaertn, F) leaves protects against sodium arsenite - induced toxicity in male wistar rats. Toxicol Rep. 2021;8:774-784.
[23] Mehrzadi S, Goudarzi M, Fatemi I, Basir Z, Malayeri A, Khalili H. Chrysin attenuates sodium arsenite-induced nephrotoxicity in rats by suppressing oxidative stress and inflammation. Tissue Cell. 2021;73:101657.
[24] Panghal A, Sathua KB, Flora SJS. Gallic acid and MiADMSA reversed arsenic induced oxidative/nitrosative damage in rat red blood cells. Heliyon. 2020;6(2):e03431.
[25] Bashir N, Ahmad SB, Rehman MU, Muzamil S, Bhat RR, Mir MUR, et al. Zingerone (4-(four-hydroxy-3-methylphenyl) butane-two-1) modulates adjuvant-induced rheumatoid arthritis by regulating inflammatory cytokines and antioxidants. Redox Rep. 2021;26(1):62-70.
[26] İleritürk M, Doğan T, Kandemir Ö. Investigation of the Effect of Berberine with Arginase Activity and Oxidant-Antioxidant Parameters on Bortezomib-Induced Spleen Injury in Rats. Kocatepe Vet J. 2021;14:6-15.
[27] İleritürk M, Doğan T, Statıcı E, Rutin'in Deltamethrin ile Mide Toksisitesi Geliştirilen Ratlarda Oksidatif Stres, Apoptoz ve İnflamasyon Parametreleri Üzerine Etkisinin Araştırılması. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi 2021;35(3): 158-165.
[28] 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.
[29] Gur 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. Turkish Journal of Nature and Science. 2022;11(3):51-57.
[30] Semis HS, Gur C, Ileriturk M, Kaynar O, Kandemir FM. Investigation of the anti-inflammatory effects of caffeic acid phenethyl ester in a model of lambda-Carrageenan-induced paw edema in rats. Hum Exp Toxicol. 2021;40(12_suppl):S721-S738.
[31] Gür C, Kandemir Ö, Kandemir FM. Evaluation of the Effects of Chrysin on Diclofenac-Induced Cardiotoxicity in Rats by the Markers of Oxidative Stress, Endoplasmic Reticulum Stress and Apoptosis. Kocatepe Vet J. 2022;15(2):151-160.
[32] Mirzaei M, Moosavi M, Mansouri E, Mohtadi S, Khodayar MJ. Diosmin exerts hepatoprotective and antihyperglycemic effects against sodium arsenite-induced toxicity through the modulation of oxidative stress and inflammation in mice. J Trace Elem Med Bio. 2023;78:127154.
[33] 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 Sport Med. 2022;50(2):486-498.
[34] Ileriturk M, Benzer F, Aksu EH, Yildirim S, Kandemir FM, Dogan T, et al. Chrysin protects against testicular toxicity caused by lead acetate in rats with its antioxidant, anti-inflammatory, and antiapoptotic properties. J Food Biochem. 2021;45(2): e13593.
[35] Çomaklı S, İleritürk M, Manavoğlu Kirman E. Protective Effects of Rutin on Oxidative DNA Damage, NF-κB-Mediated Inflammation, and Apoptosis in Colistin-Induced Testicular Damage of Rats. Turkish Journal of Nature and Science. 2020;9(2):83-90.
[36] Akbari S, Amiri FT, Naderi M, Shaki F, Seyedabadi M. Sodium arsenite accelerates D-galactose-induced aging in the testis of the rat: Evidence for mitochondrial oxidative damage, NF-kB, JNK, and apoptosis pathways. Toxicology. 2022;470:153148.
[37] 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 R. 2021;28(36):49808-49819.
[38] Buyuklu M, Kandemir FM, Ozkaraca M, Set T, Bakirci EM, Topal E, et al. Benefical effects of lycopene against contrast medium-induced oxidative stress, inflammation, autophagy, and apoptosis in rat kidney. Hum Exp Toxicol. 2015;34(5):487-496.
[39] 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.