Van Vet J, 2025, 36 (2) 132-138

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Tekin S, Aykurt F, Çınar B et al. (2025).** Investigation of the Effect of *Borago officinalis* on Bax/Bcl-2/Caspase-3 Pathways Against Spleen Toxicity Induced by Lead Acetate in Rats. *Van Vet J*, 36 (2), 132-138.
DOI: https://doi.org/10.36483/vanvetj.1683003

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Investigation of the Effect of *Borago officinalis* on Bax/Bcl-2/Caspase-3 Pathways Against Spleen Toxicity Induced by Lead Acetate in Rats

Samet TEKİN[1,*] Furkan AYKURT[1] Burak ÇINAR[2] Ömer YARDIMCI[1] Burak Batuhan LAÇİN[1] Merve BOLAT[1] İsmail BOLAT[3] Ali ÇINAR[1]

[1]*Atatürk University Faculty of Veterinary Medicine, Department of Physiology, 25100, Erzurum, Turkiye*
[2]*Atatürk University Faculty of Medicine, Department of Pharmacology, 25100, Erzurum, Turkiye*
[3]*Atatürk University Faculty of Veterinary Medicine, Department of Pathology, 25100, Erzurum, Turkiye*

Received: 25.04.2025 Accepted: 14.07.2025

ABSTRACT Lead is a highly toxic heavy metal and an environmental pollutant. Lead exposure causes damage and dysfunctions in organs. In tissues exposed to lead, ROS increase, resulting in oxidative stress, inflammation and apoptosis. In this study, we aimed to investigate the protective effects of *Borago officinalis* (BO) against lead, whose toxic effects are well-established. In this study, 40 Sprague-Dawley rats were used. The rats were randomly divided into 5 groups. The groups were determined as control, BO100, Pb (20 mg/kg), Pb+BO50 and Pb+BO100. After the experimental procedures were completed, the tissues were transported in cold chain and stored in -80 deep freezer until the experiments were performed. Keap-1 level was determined in spleen tissue using Western blot method. At the same time, IL-1β, IL-10, NF-κB, Bax, Bcl-2, Caspase-3 levels were determined by RT-PCR method. BO administration stimulated Keap-1 level in relation to oxidative stress. While BO suppressed IL-1β and NF-κB levels, it stimulated IL-10 level, which are anti-inflammatory markers. BO also suppressed Bax and Kaspas-3 levels, while significantly stimulating Bcl-2 level. With these effects, it was observed that BO had anti-oxidant, anti-inflammatory, anti-apoptotic effects in the spleen tissue of rats treated with lead acetate.

Keywords: *Apoptosis, Borago officinalis, Inflammation, Lead acetate, Oxidative stress.*

ÖZ

Ratlarda Kurşun Asetat ile İndüklenen Dalak Toksisitesine Karşı *Borago officinalis*'in Bax/Bcl-2/Kaspaz-3 yolakları üzerine Etkisinin İncelenmesi

Kurşun oldukça toksik bir ağır metal olup ve çevresel bir kirleticidir. Kurşun maruziyeti organlarda hasara ve fonksiyon bozukluklarına neden olmaktadır. Kurşuna maruz kalan dokularda ROS artışı buna bağlı, oksidatif stres, inflamasyon ve apoptosis şekillenmektedir. Biz bu çalışmada etkileri bilinen kurşuna karşı *Borago officinalis* (BO)'nun koruyucu etkilerini araştırmayı amaçladık. Bu çalışmada, 40 adet Sprague- Dawley cinsi ratlar kullanıldı. Ratlar rastgele 5 gruba ayrıldı. Gruplar kontrol, BO100, Pb (20 mg/kg), Pb+BO50 ve Pb+BO100 olarak belirlendi. Deney uygulamaları tamamlandıktan sonra, dokular soğuk zincirde taşınarak, deneylerin yapılıncaya kadar -80 derin dondurucuda saklandı. Dalak dokusunda Western Blot yöntemi kullanılarak Keap-1 düzeyi belirlendi. Aynı zamanda RT-PCR yöntemi kullanılarak IL-1β, IL-10, NF-κB, Bax, Bcl-2, Kaspaz-3 düzeyleri belirlendi. BO uygulaması oksidatif stresle ilgili olarak Keap-1 düzeyini uyardı. BO, IL-1β ve NF-κB düzeyini baskılarken, anti-inflamatuar markırlar olan IL-10 düzeyini uyardığı belirlendi. BO aynı zamanda Bax ve Kaspas-3 seviyesini baskılarken, Bcl-2 seviyesini önemli ölçüde uyarmaktadır. BO bu etkileriyle kurşun asetat uygulanan ratların dalak dokusunda anti-oksidan, anti-inflamatuar, anti-apoptotik etkilere sahip olduğu gözlendi.

Anahtar Kelimeler: *Apoptosis, Boragoofficinalis, İnflamasyon, Kurşun asetat, Oksidatif stres.*

INTRODUCTION

The spleen is the second largest lymphoid organ in the body. As such, it performs many important functions, such as hematopoiesis and the purification of red blood cells. Due to its structure, the spleen plays a role in purifying the blood of pathogens and abnormal cells. The antigen-presenting cells (APCs) it contains regulate the response of B and T lymphocytes (Lewis et al. 2019).

Lead is a naturally occurring metal found widely in the environment (Gupta 2018; Munir et al. 2022). Lead

✉ *Corresponding author: samet.tekin@atauni.edu.tr

exposure can cause both serious environmental pollution and health problems in humans. Sources of lead exposure range from food, air, soil, drinking water, industrial emissions, e-waste, plant products and domestic sources (Wang et al. 2002; Liu et al. 2013; Frank et al. 2019; Obeng-Gyasi et al. 2021).

After lead exposure, lead absorbed from the body passes into the blood and is distributed to all tissues and cells. Body tissues and cells are sensitive to lead exposure. Lead taken into the body is highly toxic for many cell types (Harshitha et al. 2024). Lead is also recognized as an enzymatic toxicant, nephrotoxic, neurotoxic and immunotoxic agent. Lead exposure causes serious damage to the spleen tissue. Since the spleen alone contains ¼ of mammalian lymphocytes, it is considered the second largest organ of the immune system (Cesta 2006). The spleen is a storage point for immune cells and a critical tissue for immune response activation and peripheral haematopoesis. It also acts as a filter for physiologically expired erythrocytes (Lewis et al. 2019). Target cells of lead exposure in spleen tissue are T cells and macrophages. Lead exposure causes problems in the functions of lymphocytes in the production of cytokines and immunoglobulins. Oxidative stress is formed in cells exposed to lead and causes imbalances between the production of free radicals and the antioxidant defence system. A study has shown that lead exposure induces oxidative stress in rats by depleting antioxidant enzymes and increasing ROS production (Dkhil et al. 2016). Nrf-2 regulates cellular responses to oxidative stress. Nrf-2 is bound by Keap-1 and remains inactive. However, while oxidative stress formed in the cell suppresses the level of Keap-1, Nrf-2 and subsequently triggers the antioxidant defence mechanism (Jiang et al. 2021).

Increased intracellular ROS and decreased antioxidant defence system lead to stimulation of lipid peroxidation in cellular components, especially phospholipids. Different inflammatory signalling pathways are triggered by this process. Humoral and cell-associated immune activities, inflammation, autoimmune reactions, susceptibility and disease resistance can be influenced by this inflammatory signalling. In the defence of the organism against an infection, many different cell types try to manage the process with complex mechanisms. Cytokines are involved in the control of this complex process. Pro-inflammatory cytokines potentiate the immune response, while anti-inflammatory cytokines suppress the immune response. These two different types of cytokines control the immune response occurring in tissues (Harshitha et al. 2024).

Apoptosis is the most fundamental pathway of cell death in the body. The regulation of apoptosis is highly complex and many molecules are involved in this process. When cells receive these signaling stimuli, they initiate regulatory procedures corresponding to these stimuli. Proapoptotic proteins and anti-apoptotic proteins control the occurrence of apoptosis in the cell by regulating the permeability of the mitochondrial membrane. The caspase family in the cell can direct the cell to apoptosis in response to incoming stimuli. Especially caspase-3 is effective and triggers apoptosis by acting on protein substrates (Li et al. 2022).

Borago officinalis linoleic acid (35-38%), oleic acid (16-20%), palmitic acid (10-11%), stearic acid (3.5-4.5%), eicosenoic acid (3.5-5.5%), and erucic acid (1.5-3.5%) (Asadi-Samani et al. 2014). *Borago officinalis* L., a plant with high medicinal and nutritional value and numerous applications, is also known as starflower and Boraginaceae. According to the results of some studies, it can be used as a supportive treatment in diabetic neuropathy, respiratory, urinary and skin disorders as well as cardiovascular and inflammatory diseases (Shannon and Graef 1992; Ruff et al. 1996). The plant material from which borage oil is obtained is grass (Boraginisherba) and seeds (Boraginis semen) Due to its high gamma-linolenic acid (GLA) content, the oil is valuable as a food and pharmaceutical raw material (Ruff et al. 1996). Phytochemical analyses showed that borage contains carbohydrates, fatty acids, phytosteroids, polyphenols (including vanillic, p-coumaric, p-hydroxybenzoic, gentisic, caffeic, sinapic, rosmarinic and chlorogenic acids, quercetin, isoramnetin and kaempferol), tannins, saponins, mucus compounds, organic acids (ascorbic, malic, citric, acetic and lactic acid), tocopherols, alantoin, mineral salts and vitamins, as well as essential oil (Pieszak et al. 2012; Karimi et al. 2018). Due to its active ingredient content, B. officinalis plant extract can be used in topical skin products with antioxidant, anti-inflammatory, anti-ageing, UV-protective, soothing or emollient effects (Asadi-Samani et al. 2014).

This study aimed to determine the anti-oxidant, anti-inflammatory and anti-apoptotic effects of *Borago officinalis* against spleen damage induced by lead acetate in rats.

MATERIAL AND METHODS

This study was approved by Atatürk University Experimental Animals Local Ethics Committee (HADYEK Decision No: 2025/59). All animal procedures were performed at Atatürk University Medical Experimental Application and Research Centre.

Chemicals

PbAc (Cas No: 6080-56-4) and borage oil (*Borago officinalis*) were purchased commercially.

Animals and Groups

In our study, 40 male adult Spraque Dawley rats weighing 220-250 grams at the age of 12 weeks were used. Animals were subjected to a 5-day adaptation period before the experiment. The rooms where the rats were maintained were set as 12 hours light and 12 hours dark cycle. The temperature (21±3 °C) and humidity (40-50%) were adjusted in the rooms where the rats were maintained. Rats had unrestricted access to water or food throughout the study.

All rats in the experimental group were randomly divided into 5 different groups with 8 animals in each group. The groups were arranged as Control, BO100, Pb, Pb+BO50, Pb+BO100.

1. 1 ml distilled water was given to the control group for 14 days.

BO100 group received Borage officinalis (i.g) at a dose of 100 mg/kg for 14 days.

3. Pb group received Pb intraperitoneally (i.p) at a dose of 20 mg/kg for 7 days.

4. Pb+BO50 group received lead acetate at a dose of 20 mg/kg for 7 days and Borage officinalis at a dose of 50 mg/kg i.g. for 14 days.

5. 20 mg/kg dose of lead acetate was administered to Pb+BO100 group for 7 days and 100 mg/kg dose of Borage officinalis was administered i.g. for 14 days.

The doses of lead acetate and *Borage officinalis* used in the studies were determined from the literature

(Ghahremanitamadon et al. 2014; Khattab et al. 2017; Al-Megrin et al. 2019).

Sample Collection

Animals were decapitated 24 hours after the last application under moderate sevoflurane anaesthesia. Spleen tissues were carefully removed and placed in liquid nitrogen. They were kept at -80 °C until molecular and western blot analyses were performed.

Gene Expression Analyses

QI Azol Lysis Reagent was used for total RNA isolation from spleen tissues of rats in all groups and the manufacturer's procedures were strictly followed. Total RNA concentrations were determined using a Nano Drop (Bio Tek Epoch) device. The iScript cDNA Synthesis Kit (Bio-Rad) was used to obtain cDNA from total RNA according to the manufacturer's instructions.

Table 1: Primer Sequences.

Gene	Accession No	Sequences (5′-3′)	Length (bp)
Bax-F	NM_017059.2	TTTCATCCAGGATCGAGCAG-3’	154
Bax-R		AATCATCCTCTGCAGCTCCA-3’	
Bcl-2-F	NM_016993.2	GACTTTGCAGAGATGTCCAG-3’	214
Bcl-2-R		TCAGGTACTCAGTCATCCAC-3’	
Caspase-3-F	NM_012922.2	ACTGGAATGTCAGCTCGCAA-3’	270
Caspase-3-R		GCAGTAGTCGCCTCTGAAGA-3’	
NF-κB -F	NM_001276711.2	AGTCCCGCCCCTTCTAAAAC-3’	106
NF-κB -R		CAATGGCCTCTGTGTAGCCC-3’	
IL-1β-F	NM_031512.2	ATGGCAACTGTCCCTGAACT	197
IL-1β-R		AGTGACACTGCCTTCCTGAA	
IL-10-F	NM_012854.2	GCCTTCAGTCAAGTGAAGAC	149
IL-10-R		GGCATCACTTCTACCAGGTA	
β-Actin-F	NM_031144.3	CAGCCTTCCTTCTTGGGTATG	360
β-Actin-R		AGCTCAGTAACAGTCCGCCT	

Primer sequences of the genes shown in Table 1 were reacted with iTaq Universal SYBR Green Supermix (BIO-RAD) kit according to the manufacturer's instructions. mRNA transcription levels were determined by Rotor-Gene Q (Qiagen). CT values given by the device were normalised to ß-Actin using the $2^{-\Delta\Delta CT}$ method.

Western Blot Analysis

The spleen tissue samples were crushed with liquid nitrogen in a mortar and pestle and powdered. The powdered samples were homogenised in RIPA buffer and centrifuged at 16000 G for 20 min. Protein levels were determined from the supernatants obtained after centrifugation by protein BCA analysis kit. Protein samples were prepared in Laemmli buffer (Tris-HCl pH 6.8, glycerol, bromophenol blue, sodium dodeacyl sulphate, 2-mercaptoethanol) and the same volume of protein samples were subjected to 10% sodium dodeacyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The samples were then transferred to nitrocellulose membranes. The membranes were washed and kept in 5% bovine albumin serum for 1 hour. β-Actin (sc-47778) and Keap-1 (sc-365626) primary antibodies were kept overnight at +4 degrees Celsius. Then, the secondary antibody was washed again and incubated at 37 °C for 90 min. Densitometric analysis was performed at least 3 times using Biorat Vlarity Max ECL substrate in Image Lab programme to visualise the bands formed on the membrane (Kandemir et al. 2020; Simsek et al. 2024).

Statistical Analysis

All data obtained were analysed using IBM SPSS software. Data were presented as mean±standard deviation and Tukey post hoc tests and one-way analysis of variance (ANOVA) were used for multiple comparisons. Statistical difference was determined at $p<0.05$, $p<0.01$ and $p<0.001$ levels.

RESULTS

Effects of *Borago officinalis* on Keap-1 Protein Level in Lead Acetate-Induced Spleen Injury

Keap-1 protein levels were determined using WB method to better understand oxidative stress levels. Keap-1 protein level was significantly higher in the BO100 group compared to the control group ($p<0.05$). Keap-1 protein levels obtained from Pb and Pb+BO50 groups were quite close to each other, however; there was a statistical difference between them and the control ($p<0.001$). The results obtained from the Pb+BO100 group were statistically different from the Pb group, but still statistically different from the control ($p<0.01$, Figure 1).

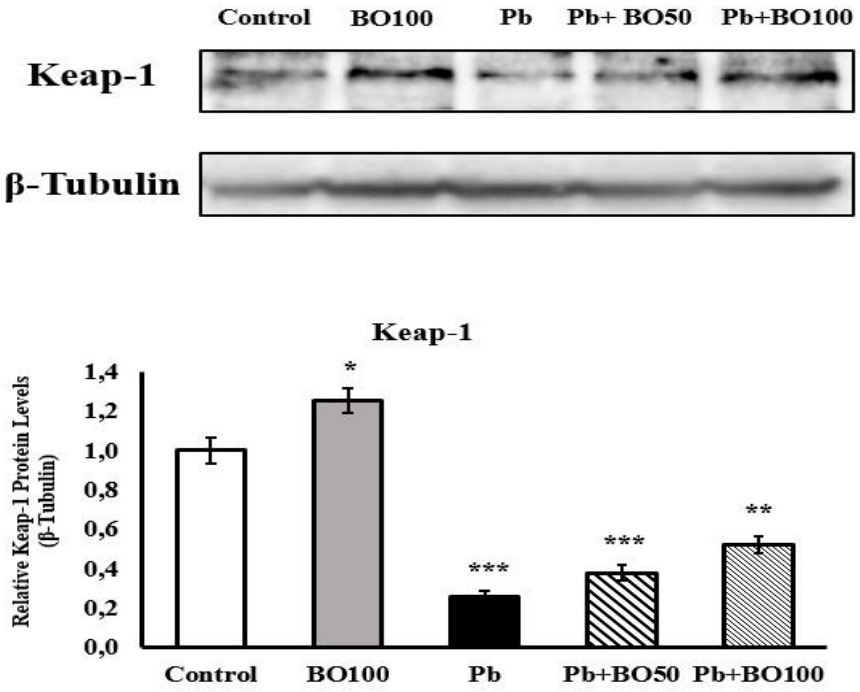

Figure 1: Effects of Pb and BO administrations on Keap-1 protein levels in spleen tissue of rats. Values are given as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

Effects of *Borago officinalis* on IL-1β, IL-10 and NF-κB Inflammatory Gene Expression Levels in Spleen Injury Induced by Lead Acetate

IL-1β, IL-10 and NF-κB mRNA transcript levels were determined in spleen tissues of rats exposed to lead acetate. In the spleen tissue, IL-1β level and transcription factor NF-κB, which are among the pro-inflammatory cytokines, were significantly increased in the lead-exposed groups compared to the control, while anti-inflammatory IL-10 level was significantly decreased (p<0.001). Although IL-1β and NF-κB levels in the spleen tissue of the Pb+BO50 groups were lower than in the Pb-treated group (p<0.01), this decrease was stronger in the Pb+BO100 groups and the statistical difference between them and the control was reduced to the lowest level (p<0.05, Figure 2).

Among the anti-inflammatory cytokines, IL-10 level was higher in the Pb+BO50 group compared to the Pb group (p<0.01), this value was much higher in the Pb+BO100 group and there was little statistical difference between them and the control group (p<0.05). IL-1β, IL-10 and NF-κB mRNA transcript levels in the control and BO100 groups were very close to each other and there was no statistical difference between them (p>0.05).

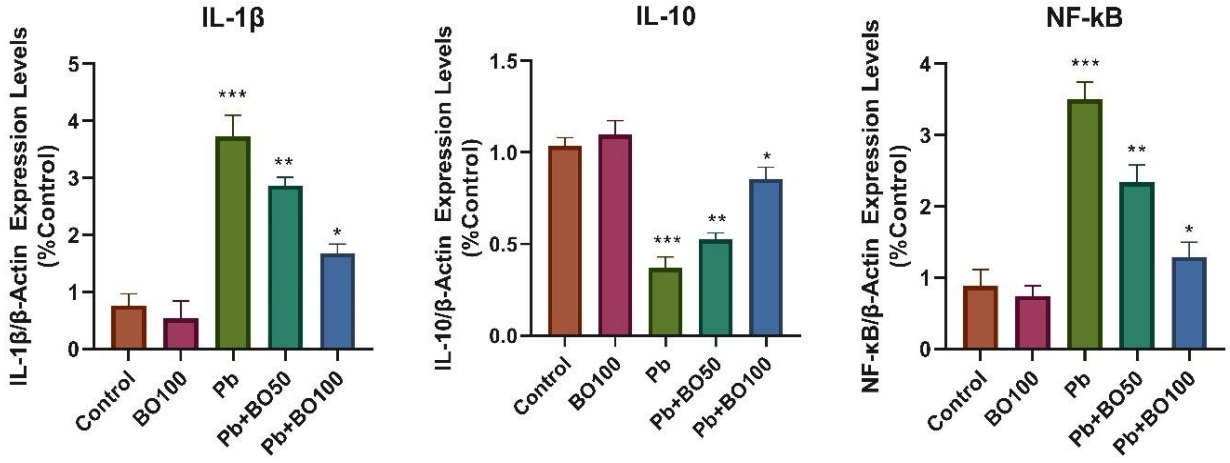

Figure 2: Spleen tissue IL-1β, IL-10, NF-κB, relative mRNA transcript levels after Pb and BO applications to rats. Statistical significance; Control and others: *p<0.05, **p<0.01, ***p<0.001.

Effects of *Borago officinalis* on Bax, Bcl-2 and Caspase-3 Apoptotic Gene Expression Levels in Spleen Injury Induced by Lead Acetate

Bax, Bcl-2 and Caspase-3 mRNA transcript levels were determined in spleen tissues of rats exposed to lead acetate. Bax and Caspase-3 levels, which are among the apoptotic markers, were significantly higher in Pb treated groups compared to the control ($p<0.001$). When Bax and Caspase-3 levels obtained from Pb+BO50 treated groups were compared with the control, it was observed that there was still a statistical difference between them ($p<0.01$). Bax and Caspase-3 levels in the spleen tissue of the Pb+BO100 treated groups were much lower than the Pb group and the statistical difference between them and the control was very low ($p<0.05$, Figure 3).

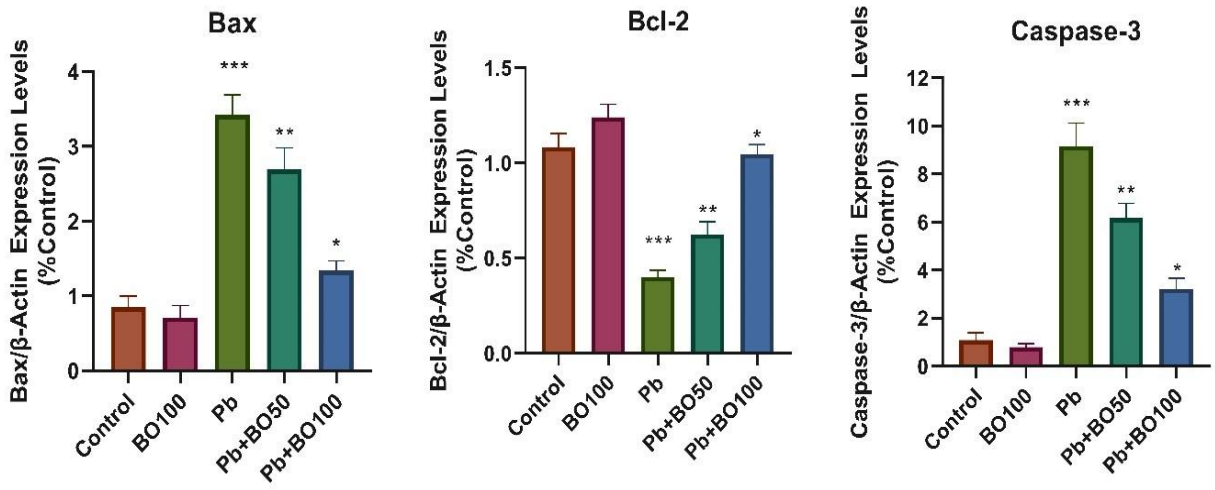

Figure 3: Spleen tissue Bax, Bcl-2, Caspase-3, relative mRNA transcript levels after Pb and BO applications to rats. Statistical significance; Control and others: *p<0.05, **p<0.01, ***p<0.001.

When the Bcl-2 levels obtained from the Pb treated groups were compared with the control, it was determined that there was a statistically significant difference between them ($p<0.001$). It was observed that the anti-inflammatory Bcl-2 level was higher in the Pb+BO50 group than in the Pb group, but there was a statistically high difference between them and the control ($p<0.01$). Bcl-2 levels obtained from the Pb+BO100 groups were significantly higher than the Pb group, but there was still a statistical difference between them and the control group ($p<0.05$). Bax, Bcl-2 and Caspase-3 levels obtained from control and BO100 groups were very close to each other and there was no statistical difference between them ($p>0.05$).

DISCUSSION AND CONCLUSION

Lead acetate has very high toxic properties and causes many pathologies in humans and animals (Abdelhamid et al. 2020). Lead acetate-induced toxicities are becoming quite common in the world with increasing industrialization (Abubakar et al. 2019). Plant-based solutions to these conditions may be promising and there is an increasing number of studies on this subject (Kucukler et al. 2021). Borago officinalis contains a significant amount of fatty acids. Gamma linoleic acid (GLA) is present in high amounts, as well as linoleic acid, stachydonic acid, phenolic acids, and flavonoids. Of these substances, gamma linoleic acid, in particular, is formed in the small intestine after oral intake. Its bioavailability in the phospholipid or triglyceride form is quite high compared to free fatty acids. Peak plasma levels are reached 1 to 3 hours after ingestion. Gamma linoleic acid is metabolized by elongase to dihomo-γ-linolenic acid (DGLA). DGLA, in turn, is converted to PGE1, which is crucial in this anti-inflammatory process. Once its function is completed in the body, GLA is eliminated from the body through beta-oxidation. Borago officinalis, which has particularly potent antioxidant and anti-inflammatory properties, is thought to exert these effects through GLA. Of course, other active ingredients may also contribute to these effects. (Monari et al. 2025). Officinalis, also known as borage oil, has strong antioxidant, antiapoptotic and anti-inflammatory properties (Lukivskaya et al. 2012). In this study, we investigated the effects of *Borago officinalis* against oxidative, inflammatory and apoptotic damage in spleen tissue of rats treated with lead acetate using Western Blot and RT-PCR methods.

ROS-induced oxidative stress is the first of the problems shaped in tissues and cells after lead acetate exposure (Alhusaini et al. 2019). Oxidative stress is characterized by an increase in the amount of ROS in cells and a decrease in the level of antioxidant enzymes (Aksu et al. 2021). Keap1/Nrf2 is a key pathway for oxidative stress (Yu et al. 2019). In order to determine the effects of this pathway and to determine whether oxidative stress occurs in tissues, we determined the level of Keap-1 using Western Blot method. Nrf2 protects the cell against toxic agents and carcinogens and increases the cytoprotective effect (Cheng et al. 2019). Keap1 has the ability to negatively regulate the transcription level of Nrf2 (Jaramillo and Zhang 2013). And we found that Keap-1 level decreased in the spleen tissue of lead-exposed rats. Nrf-2s that become free pass to the nucleus as a continuation of the pathway and form a heterodimer with a small MAF (sMAF).(Itoh et al. 1997). This heterodimer structure then binds to the antioxidant reaction element (ARE) leading to the production of numerous cytoprotective agents (Friling et al. 1990). This association is consistent with oxidative stress caused by peroxide accumulation (Ye et al. 2016). This pathway is

directly related to oxidative stress. In a study, it was observed that lead application induced oxidative stress in oocytes, increased Nrf-2 level and suppressed Keap-1 level (Jiang et al. 2021). In order to understand whether this pathway works or not, we performed an experiment on Keap-1 level and our results showed that Keap-1 inhibition occurred after lead exposure. Especially the high dose of our BO application significantly increased the Keap-1 level and showed that it is important in controlling oxidative stress by suppressing the pathway again.

Inflammation is caused by an imbalance between anti-inflammatory and pro-inflammatory cytokines and proteins that may be associated with inflammation (He et al. 2017). Not only protein and inflammatory markers but also transcription factors such as NF-κB and p53 can cause inflammation (He et al. 2017). NF-κB accelerates the inflammatory process by stimulating genes responsible for the expression of inflammatory cytokines such as IL-1β and TNF-α (Akaras et al. 2023). IL-10 is an anti-inflammatory cytokine and inhibits the production of pro-inflammatory cytokines mainly from T cells, macrophages and dentritic cells (Bamboat et al. 2010). Many studies have shown that IL-10 levels are suppressed in tissues of experimental animals treated with lead acetate, while proinflammatory cytokines and transcription factors are stimulated (Abbaszadeh et al. 2021; Dahran et al. 2025). In our study, we determined that lead acetate administration to rats increased IL-1β and NF-κB levels in the spleen tissue and suppressed IL-10 levels. Administration of high dose of BO caused suppression of IL-1β, a pro-inflammatory cytokine, and NF-κB, a transcription factor, in the spleen tissue, while IL-10, an anti-inflammatory cytokine, increased significantly.

Bax is a proapoptotic protein and plays a central role in mitochondria-dependent apoptosis. While Bcl-2 is involved in the regulation of programmed cell death, it can ensure cell survival with its anti-apoptotic genes. Caspase-3 has an apoptotic effect and is activated by caspase-9 (Simsek et al. 2016). Caspase-3 activation leads to proteolytic degradation and cell death (Kuzu et al. 2018). In a study, it was observed that Bax and Caspase-3 levels increased and Bcl-2 level decreased in liver, kidney and testicular tissues of rats administered lead (Bidanchi et al. 2022). In our study, it was determined that Bax and Caspase-3 levels increased in the spleen tissue of rats treated with lead compared to the control. It was observed that BO administration, especially the high dose of BO, significantly suppressed the levels of Bax and Caspase-3 proteins. Lead treatment significantly reduced Bcl-2 expression in spleen tissue. We determined that BO application was important in increasing the anti-apoptotic Bcl-2 level and obtaining values close to the control.

In conclusion, lead exposure in rats is significantly toxic and causes damage in many tissues such as spleen, liver, kidney, brain and testis. Increased ROS in the cell after lead exposure stimulates oxidative stress. We determined Keap-1 level in order to determine whether the pathway is stimulated or not. While Pb administration significantly suppressed Keap-1 level, BO100 administration together with Pb caused an increase in Keap-1 level. In addition, IL-1β, NF-κB, IL-10 levels were determined to evaluate inflammatory processes in spleen tissue. Pb administration increased IL-1β and NF-κB levels and decreased IL-10 levels. BO application together with Pb was found to be very important in improving these levels close to the control level. Bax, Bcl-2 and Caspase-3 levels were determined by RT-PCR method to determine whether the inflammatory process was followed by an apoptotic signal. Pb treatment stimulated Bax and Caspase-3 levels, while Bcl-2 level was suppressed. The use of BO together with Pb led to suppression of proapoptotic markers (Bax and Caspase-3) and stimulation of anti-apoptotic (Bcl-2) levels. It was determined that oxidative stress, inflammation and apoptotic pathways were stimulated in the spleen tissue of rats exposed to Pb, and BO administration showed a protective effect with anti-oxidant, anti-inflammatory and anti-apoptotic effects in rats.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: ST, FA
Supervision / Consultancy: ST, BÇ, AÇ
Data Collection and / or Processing: ST, BBL, ÖY
Analysis and / or Interpretation: MB, İB
Writing the Article: ST, AÇ
Critical Review: ST, AÇ

REFERENCES

Abbaszadeh S, Yadegari P, Imani A, Taghdir M (2021). Vitamin D3 protects against lead-induced testicular toxicity by modulating Nrf2 and NF-kappaB genes expression in rat. *Reprod Toxicol*, 103, 36-45.

Abdelhamid FM, Mahgoub HA, Ateya AI (2020). Ameliorative effect of curcumin against lead acetate-induced hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. *Environ Sci Pollut Res Int*, 27, 10950-65.

Abubakar K, Muhammad Mailafiya M, Danmaigoro A et al. (2019). Curcumin Attenuates Lead-Induced Cerebellar Toxicity in Rats via Chelating Activity and Inhibition of Oxidative Stress. *Biomolecules*, 9.

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

Aksu EH, Kandemir FM, Kucukler S (2021). Ameliorative effect of hesperidin on streptozotocin-diabetes mellitus-induced testicular DNA damage and sperm quality degradation in Sprague-Dawley rats. *J Food Biochem*, 45, e13938.

Al-Megrin WA, Alkhuriji AF, Yousef AOS et al. (2019). Antagonistic Efficacy of Luteolin against Lead Acetate Exposure-Associated with Hepatotoxicity is Mediated via Antioxidant, Anti-Inflammatory, and Anti-Apoptotic Activities. *Antioxidants (Basel)*, 9.

Alhusaini AM, Faddah LM, Hasan IH et al. (2019). Vitamin C and Turmeric Attenuate Bax and Bcl-2 Proteins' Expressions and DNA Damage in Lead Acetate-Induced Liver Injury. *Dose Response*, 17, 1559325819885782.

Asadi-Samani M, Bahmani M, Rafieian-Kopaei M (2014). The chemical composition, botanical characteristic and biological activities of *Borago officinalis*: a review. *Asian Pac J Trop Med*, 7 (1), 22-28.

Bamboat ZM, Ocuin LM, Balachandran VP et al. (2010). Conventional DCs reduce liver ischemia/reperfusion injury in mice via IL-10 secretion. *J Clin Invest*, 120, 559-569.

Bidanchi RM, Lalrindika L, Khushboo M et al. (2022). Antioxidative, anti-inflammatory and anti-apoptotic action of ellagic acid against lead acetate induced testicular and hepato-renal oxidative damages and pathophysiological changes in male Long Evans rats. *Environ Pollut*, 302, 119048.

Cesta MF (2006). Normal structure, function, and histology of the spleen. *Toxicol Pathol*, 34, 455-465.

Cheng Q, Jiang S, Huang L et al. (2019). Zearalenone induced oxidative stress in the jejunum in postweaning gilts through modulation of the Keap1-Nrf2 signaling pathway and relevant genes1. *J Anim Sci*, 97, 1722-1733.

Dahran N, Alobaidy MA, Owaydhah WH et al. (2025). Polydatin Mitigates Lead-Induced Nephropathy by Modulating Oxidative Stress, Inflammation, and the AMPK/AKT/Nrf2 Pathway in Rats. *Biol Trace Elem Res*, DOI: 10.1007/s12011-025-04570-9

Dkhil MA, Moneim AE, Al-Quraishy S (2016). Indigofera oblongifolia Ameliorates Lead Acetate-Induced Testicular Oxidative Damage and Apoptosis in a Rat Model. *Biol Trace Elem Res*, 173, 354-361.

Frank JJ, Poulakos AG, Tornero-Velez R, Xue J (2019). Systematic review and meta-analyses of lead (Pb) concentrations in environmental media (soil, dust, water, food, and air) reported in the United States from 1996 to 2016. *Sci Total Environ*, 694, 133489.

Friling RS, Bensimon A, Tichauer Y, Daniel V (1990). Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element. *Proc Natl Acad Sci U S A*, 87, 6258-6262.

Ghahremanitamadon F, Shahidi S, Zargooshnia S et al. (2014). Protective effects of *Borago officinalis* extract on amyloid beta-peptide(25-35)-induced memory impairment in male rats: a behavioral study. *Biomed Res Int*, 2014, 798535.

Gupta RC (2018). Veterinary Toxicology Basic and Clinical Principles Third Edition Preface. *Veterinary Toxicology: Basic and Clinical Principles, 3rd Edition*, Xxxi-Xxxii.

Harshitha P, Bose K, Dsouza HS (2024). Influence of lead-induced toxicity on the inflammatory cytokines. *Toxicology*, 503, 153771.

He Y, Tan D, Mi Y, Zhou Q, Ji S (2017). Epigallocatechin-3-gallate attenuates cerebral cortex damage and promotes brain regeneration in acrylamide-treated rats. *Food Funct*, 8, 2275-2282.

Itoh K, Chiba T, Takahashi S et al. (1997). An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun*, 236, 313-322.

Jaramillo MC, Zhang DD (2013). The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev*, 27, 2179-2191.

Jiang X, Xing X, Zhang Y et al. (2021). Lead exposure activates the Nrf2/Keap1 pathway, aggravates oxidative stress, and induces reproductive damage in female mice. *Ecotoxicol Environ Saf*, 207, 111231.

Kandemir FM, Yildirim S, Kucukler S et al. (2020). Protective effects of morin against acrylamide-induced hepatotoxicity and nephrotoxicity: A multi-biomarker approach. *Food Chem Toxicol*, 138, 111190.

Karimi E, Oskoueian E, Karimi A, Noura R, Ebrahimi M (2018). L. flower: a comprehensive study on bioactive compounds and its health-promoting properties. *Journal of Food Measurement and Characterization*, 12, 826-38.

Khattab HAH, Abdallah IZA, Yousef FM, Huwait EA (2017). Efficiency of Borage Seeds Oil against Gamma Irradiation-Induced Hepatotoxicity in Male Rats: Possible Antioxidant Activity. *Afr J Tradit Complement Altern Med*, 14, 169-79.

Kucukler S, Benzer F, Yildirim S et al. (2021). 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*, 199, 1501-14.

Kuzu M, Kandemir FM, Yildirim S et al. (2018). Morin attenuates doxorubicin-induced heart and brain damage by reducing oxidative stress, inflammation and apoptosis. *Biomed Pharmacother*, 106, 443-453.

Lewis SM, Williams A, Eisenbarth SC (2019). Structure and function of the immune system in the spleen. *Sci Immunol*, 4 (33), e:eaau6085.

Li L, Wang S, Zhou W (2022). Balance Cell Apoptosis and Pyroptosis of Caspase-3-Activating Chemotherapy for Better Antitumor Therapy. *Cancers (Basel)*, 15 (1), 26.

Liu J, Li L, Wang Y, Yan C, Liu X (2013). Impact of low blood lead concentrations on IQ and school performance in Chinese children. *PLoS One*, 8, e65230.

Lukivskaya OY, Naruta E, Sadovnichy V, Kirko S, Buko VU (2012). Reversal of experimental ethanol-induced liver steatosis by borage oil. *Phytother Res*, 26, 1626-1631.

Monari S, Ferri M, Zappi A et al. (2025). Bioaccessibility and Biological Activities of Phytochemicals from Wild Plant Infusions and Decoctions Before and After Simulated In Vitro Digestion. *Plant Foods Hum Nutr*, 80, 81.

Munir N, Jahangeer M, Bouyahya A et al. (2022). Heavy Metal Contamination of Natural Foods Is a Serious Health Issue: A Review. *Sustainability*, 14.

Obeng-Gyasi E, Roostaei J, Gibson JM (2021). Lead Distribution in Urban Soil in a Medium-Sized City: Household-Scale Analysis. *Environ Sci Technol*, 55, 3696-3705.

Pieszak M, Mikolajczak PL, Manikowska K (2012). Borage (*Borago officinalis* L.)-a valuable medicinal plant used in herbal medicine. *Herba Polonica*, 58.

Ruff HA, Markowitz ME, Bijur PE, Rosen JF (1996). Relationships among blood lead levels, iron deficiency, and cognitive development in two-year-old children. *Environ Health Perspect*, 104, 180-185.

Shannon MW, Graef JW (1992). Lead intoxication in infancy. *Pediatrics*, 89, 87-90.

Simsek H, Demiryürek S, Demir T et al. (2016). 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. *IJBMS*, 19, 209-214.

Simsek H, Gur C, Kucukler S et al. (2024). Carvacrol Reduces Mercuric Chloride-Induced Testicular Toxicity by Regulating Oxidative Stress, Inflammation, Apoptosis, Autophagy, and Histopathological Changes. *Biol Trace Elem Res*, 202, 4605-4617.

Wang CL, Chuang HY, Ho CK et al. (2002). Relationship between blood lead concentrations and learning achievement among primary school children in Taiwan. *Environ Res*, 89, 12-18.

Ye F, Li X, Li L, Yuan J, Chen J (2016). t-BHQ Provides Protection against Lead Neurotoxicity via Nrf2/HO-1 Pathway. *Oxid Med Cell Longev*, 2016, 2075915.

Yu L, Li HX, Guo JY et al. (2019). Di (2-ethyl hexyl) phthalate (DEHP)-induced spleen toxicity in quail (Coturnix japonica) via disturbing Nrf2-mediated defense response. *Environ Pollut*, 251, 984-989.