ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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ANKAFERD BLOOD STOPPER ALLEVIATES CADMIUM-INDUCED LUNG INJURY BY REDUCING MITOCHONDRIAL STRESS-RELATED APOPTOSIS VIA BAX/BCL-2 AND CYT-C/CAS-3 PATHWAYS

ANKAFERD BLOOD STOPPER BAX/BCL-2 VE CYT-C/CAS-3 YOLAKLARI ARACILIĞIYLA MİTOKONDRİYAL STRESLE İLİŞKİLİ APOPTOZU AZALTARAK KADMİYUM KAYNAKLI AKCİĞER HASARINI HAFİFLETİR

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Öz

Amaç
Çevresel faktörlerin bir sonucu olarak vücuda alınan kadmiyum (Kd) akciğer dokularında inflamasyon, oksidatif stres ve artan apoptozis ile hasara neden olur. Hemostatik bir ajan olarak kullanılan Ankaferd Blood Stopper (ABS), bileşimindeki beş farklı bitki özü ve bileşeni nedeniyle antioksidan, antienflamatuar, antibakteriyel, antiapoptotik ve yara iyileştirici özelliklere sahiptir. Bu nedenle çalışmamızda Kd'un akciğer üzerinde oluşturduğu toksisite üzerine ABS'nin iyileştirici etkisini araştırmayı amaçladık.

Gereç ve Yöntem
Çalışmada 32 rat kullanıldı ve her grupta 8 rat olmak üzere 4 gruba ayrıldı: kontrol, Kd (2,5 mg/kg tek doz ip), ABS (1,5 ml/kg tek doz ip) ve Kd+ABS (Kd, 2,5 mg/kg tek doz ip-ABS, 1,5 ml/kg tek doz ip). Akciğer dokuları histopatolojik olarak değerlendirildi. İnflamasyon, tümör nekroz faktörü-α (TNF-α) ile immünohistokimyasal olarak değerlendirildi. Oksidatif stres, total oksidan seviye (TOS) ve total antioksidan seviye (TAS) ile spektrofotometrik yöntem kullanılarak değerlendirildi. Apopitoz, Bcl-2 ile ilişkili X (Bax), B-hücreli lenfoma 2 (Bcl-2), Sitokrom c (Sit c) ve kaspaz 3 genlerinin rölatif mRNA kat değişimleri ile RT-PCR yöntemi kullanılarak değerlendirildi.

Bulgular
Kd grubunda, konjesyon, hemoraji ve mononükleer hücre infiltrasyonu gibi histopatolojik bulgularda artış bulundu. Kd'un TNF-α'yı yükselterek inflamasyonu arttırdığı, TOS ve OSİ artırıp TAS'ı azaltarak oksidatif stres artışına neden olduğu ayrıca Bax, Sit c ve kaspaz 3 gen ekspresyonlarının arttırıp Bcl-2 gen ekspresyonunu azaltarak mitokondriyal stres ile ilişkili apoptozise neden olduğu bulundu ($p<0.05$). ABS uygulamasından sonra tüm bu parametrelerde anlamlı düzelme olduğu belirlendi ($p<0.05$).

Sonuç
Sonuç olarak, ABS'nin Kd kaynaklı akciğer toksisitesinden koruyucu alternatif bir seçenek olabileceği ve bu konuda daha fazla araştırma yapılması gerektiği kanısındayız.

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Anahtar Kelimeler: Akciğer, Ankaferd Blood Stopper, Apoptoz, İnflamasyon, Kadmiyum, Oksidatif stres

Abstract

Objective
As a result of environmental factors, cadmium (Cd) taken into the body causes damage to lung tissues through inflammation, oxidative stress, and increased apoptosis. Ankaferd Blood Stopper (ABS), which is used as a hemostatic agent, has antioxidant, anti-inflammatory, antibacterial, antiapoptotic, and wound healing properties due to five different plant extracts and components in its composition. Therefore, in our study, we aimed to investigate the curative effect of ABS on the toxicity of Cd on the lung.

Material and Method
Thirty two rats were used in the study, and they were divided into 4 groups, with 8 rats in each group: control, Kd (2.5 mg/kg single dose ip), ABS (1.5 ml/kg single dose ip), and Kd+ABS (Kd, 2,5 mg/kg single dose ip-ABS, 1.5 ml/kg single dose ip). Lung tissues were evaluated histopathologically. Inflammation was evaluated immunohistochemically with tumor necrosis factor-α (TNF-α). Oxidative stress was evaluated with the total oxidant level (TOS) and total antioxidant level (TAS) using the spectrophotometric method. Apoptosis was evaluated using RT-PCR with relative mRNA fold changes of Bcl-2-associated X (Bax), B-cell lymphoma 2 (Bcl-2), cytochrome c (Cyt c), and caspase 3 genes.

Results
Histopathological findings such as congestion, hemorrhage, and mononuclear cell infiltration were found to increase in the Cd group. It was found that Cd increased inflammation by increasing TNF-α, increasing TOS and OSI, and decreasing TAS, causing an increase in oxidative stress. ($p<0.05$). It was determined that there was a significant improvement in all these parameters after the ABS application ($p<0.05$).

Conclusion
In conclusion, we suggested that ABS may be an alternative option to protect against Cd-induced lung toxicity, and more research should be done on this subject.

Keywords: Ankaferd Blood Stopper, Apoptosis, Cadmium, İnflammation, Lung, Oxidative stress

Introduction

Heavy metals formed as a consequence of a rise in industrial goods are regarded as one of the primary sources of environmental pollution and are known to cause a variety of health concerns (1). Cadmium (Cd), which is in the group of non-essential and heavy metals discharged to the environment in various ways, is one of the most prominent causes of environmental pollutants and is present in phosphate fertilizers, cigarettes, batteries, detergents, and refined petroleum derivatives (2, 3).

Exposure to hazardous Cd is through contaminated water, air, food, and cigarette smoke (4). It has been shown that Cd has a function in the pathophysiological process in organs such as the lungs, kidneys, liver, and bone (5). Among these organs, the lungs are the most sensitive to cadmium toxicity when taken into the body by inhalation. When cadmium is inhaled, it causes lung damage, bronchial and pulmonary inflammation, which can lead to pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), emphysema, and even lung cancer (6).

Studies have shown that the most important mechanism of cadmium toxicity is increased reactive oxygen species (ROS) load and increased oxidative stress by Cd binding to glutathione and sulfhydryl groups in cells (7, 8). It's been reported that free Cd causes oxidative stress via binding to some of the electron transport chain complexes, and some dehydrogenase enzymes in mitochondria, then inducing the activities of other oxidant enzymes such as NADPH oxidase, causing an increase in ROS (9, 10). The loss of membrane potential after mitochondrial damage leads to the release of Cytochrome c (Cyt-c) and activation of the caspase-dependent pathway, leading the cell to apoptosis (11). Increasing ROS causes a decrease in antioxidant enzymes and activates apoptotic pathways by causing damage to structural components of the cell such as DNA, protein and lipid (12–14). Cd also induces genotoxicity through DNA methylation and histone acetylation, causing a decrease in the expression of tumor suppressor genes and an increase in proinflammatory cytokines such as Interleukin-6 (IL-6), Interleukin 1 β (IL-1β) and Tumor Necrosis Factor-α (TNF-α) (15). Bax and Bcl 2 are also well-known proteins that play significant roles in

the development of oxidative stress and mitochondria-mediated apoptosis (16).

Ankaferd Blood Stopper (ABS), which is widely used as a hemostatic agent in traditional medicine, is a mixture used by standardizing the extracts of five plants such as Thymus vulgaris (thyme) dried herb (5 mg/dl), Alpinia officinarum (galangal) dried leaf (7 mg/dl), Vitis vinifera (grape) dried leaf (8 mg/dl), Glycyrrhiza glabra (licorice) dried leaf (9 mg/dl) and Urtica dioica (nettle) root (6 mg/dl) (17–19). Each of these plants in the mixture has different healing effects at the cellular and tissue level (eg cell proliferation, endothelium, angiogenesis, antioxidant, etc.) (20, 21). In addition, studies have revealed that ABS reduces the apoptotic process that occurs due to hemorrhagic and mitochondrial stress (22–24).

As a result of the literature studies, no studies were found on the antioxidant, antiapoptotic, and healing properties of ABS on the damage caused by Cd toxicity in the lung tissues. In light of all of this knowledge, an investigation into the protective effects of ABS against Cd-induced acute lung damage was undertaken.

Material and Method

Experimental Animal Protocol

Thirty-two male rats were randomly divided into 4 groups, with 8 in each group. Rats were kept in the light for 12 hours and in darkness for 12 hours at 22-24 degrees to ensure standardization Rats were fed with ad libutium in Euro type 4 cages.

Control group (n=8): 1 ml of saline was administered intraperitoneally (ip) into the rats' right inguinal region on the day of the experiment.

ABS group (n=8): 1.5 ml/kg ABS was administered ip from the right inguinal regions of the rats (25).

Cd group (n=8): Cadmium chloride ($CdCl_2$) dissolved in saline at a dose of 2.5 mg/kg was administered ip from the right inguinal region (26).

ABS + Cd group (n=8): First administered $CdCl_2$ at a single dose of 2.5 mg/kg (ip) from the right inguinal region. One hour after the cadmium application, 1.5 ml/kg ABS was administered to the rats from the same area as i.p.

24 hours after Cd administration, the rats were sacrificed under anesthesia and their lung tissues were taken. The right lung was placed in 10% neutral formaldehyde for histopathological and immunohistochemical evaluations. The left lung was wrapped in aluminum foil and stored at -80º C for biochemical and genetic analysis.

Histopathological and Immunohistochemical Analyzes

Lung tissue samples to be used for histopathological examinations were fixed with formalin and embedded in paraffin blocks. Five microns (µ) of sections were taken from the paraffin blocks with a Leica brand cylinder microtome, and the general characteristics of the lung tissue were revealed by staining with Hematoxylin+Eosin (H+E) dye and visualized on the ZEISS AX10 Lab.A1 photomicroscope 40X. Histopathological findings were evaluated and scored as “(0) = normal, (1) = mild, (2) = moderate, (3) = severe” (27).

For immunohistochemical examinations, 5 µm thick sections were taken from the lung tissue and deparaffinization was performed. Sections were stained immunohistochemically with TNF-α (Catalog no: bs-2081R, Bioss) according to the kit manufacturer's protocol. Then, immunohistochemistry analyzes were performed using secondary antibodies and evaluated by scoring on a 20X ZEISS AX10 Lab. A1 photomicroscope.

Biochemical Analyzes

Lung tissues were homogenized with phosphate buffer (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl, pH 7.4) and centrifuged. TAS (mmol Trolox Eq/L) and TOS (µmol H_2O_2 Eq/L) measurements from the supernatants were performed using colorimetric kits (Randox Laboratories, United Kingdom) by the method developed by Erel et al. in a Beckman Coulter AU5800 device (Beckman Coulter, USA). The formula ($OSI = [(TOS / (TAS) \times 100]$) was used to calculate the OSI values (28, 29).

Genetic Analyzes

RNA isolation

RNA isolation of lung samples was performed with the RiboEx (TM) RNA Isolation Kit (Atlas Biotechnology, Turkey). The amount and purity of the RNAs obtained were measured with the BioSpec nanodrop (Shimadzu Ltd., Kyoto, Japan) device. Each isolated RNA sample was standardized at 1 µg and stored at -80°C for use in the cDNA synthesis step.

cDNA Synthesis

cDNA synthesis was carried out in a thermal cycler according to the protocol of the A.B.T. TM cDNA

Table 1 Primary sequences of genes and product size

Genes	Primary sequence	product size
GAPDH (HouseKeeping)	F: AGGTTGTCTCCTGTGACTTC	130 bp
	R: CTGTTGCTGTAGCCATATTC	
Cyt c	F: TAAATATGAGGGTGTCGC	192 bp
	R: AAGAATAGTTCCGTCCTG	
Caspase 3	F: GGCCGACTTCCTGTATGCTT	110 bp
	R: CGTACAGTTTCAGCATGGCG	
Bax	F: AGGGTGGCTGGGAAGGC	93 bp
	R: TGAGCGAGGCGGTGAGG	
Bcl2	F: ATCGCTCTGTGGATGACTGAGTAC	134 bp
	R: AGAGACAGCCAGGAGAAATCAAA	

F: Forward, R: Reverse, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase, Cyt c: Cytochrome, Bax: Bcl2 associated X, protein, BCL2: B-cell lymphoma 2

Synthesis Kit (Atlas Biotechnology, Turkey). The following concentrations were prepared on ice for each sample: 10X reaction buffer 2 µl, dNTP mix (2.5 mM) 1 µl, Random hexamer (50 µM) 2 µl, Reverse Transcriptase (200 U/µl) 1 µl, RNase inhibitor 0.5 µl, RNase-free water 3.5 µl, and RNA sample 10 µl. The prepared mixture was placed in the thermal cycler and the kit protocol was used. All stages were performed in 1 cycle and obtained cDNAs were stored at -20 °C.

qRT PCR

Primer designs were made by detecting specific mRNA sequences and testing possible primer sequences using the NCBI website. The genes and specific primer sequences used in the expression step are given in Table 1. Expression levels were measured by the Biorad CFX96 (California, USA) real-time qPCR instrument using the A.B.T. 2X qPCR SYBR-Green MasterMix (Atlas Biotechnology, Turkey). RT-qPCR conditions according to the manufacturer's protocol were pre-denaturation at 95 °C for 5 min, followed by 40 cycles of 20 s at 95 °C and 30 s at 60 °C. Expression of the GAPDH gene was used for normalization and each sample was run in triplicate.

Statistical Analysis

The normality distribution was performed using the Shapiro-Wilk test. One-Way ANOVA (posthoc Bonferroni) test was used to compare data between groups. The data in our study were presented as the mean±standard deviation. All analyzes were performed using the SPSS 20 program and $p < 0.05$ was considered significant.

Results

Histopathological and immunohistochemistry findings Scores obtained after H-E staining lung tissue slices were assessed. No histopathological findings were found in the rat lung tissues in the control group. A significant increase was found in histopathological findings such as mononuclear cell infiltration, hemorrhage and congestion in the vessels, and thickening of the alveolar walls in the lung tissues of the Cd group ($p<0.001$). Similar to the control group, histopathological findings were not observed in the ABS group ($p>0.05$). Only minor signs of hemorrhage and congestion were detected in the vessels ($p=0.07$). In the Cd+ABS group, histopathological findings such as cell mononuclear cell infiltration, vascular hemorrhage and congestion, and alveolar wall thickening were observed to be significantly reduced compared to the Cd-administered group ($p<0.05$) (Figure 1).

TNF-α was evaluated immunohistochemically. TNF-α synthesis was significantly increased in the Cd group compared to the control group ($p<0.001$). TNF-α synthesis was found to be significantly decreased in the Cd+ABS group compared to the Cd group ($p<0.001$) (Table 2) (Figure 2).

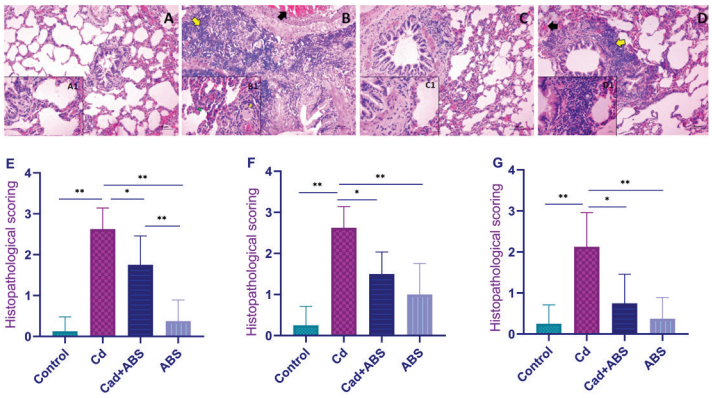

Figure 1;
A and A1 images contain H-E staining images of the control group. There is no histopathological finding in the images of the control group. B and B1 images belong to the Cd group and the black arrow shows the congestion and hemorrhage in the vessels, while the mononuclear cell infiltration is marked with the yellow arrow. C and C1 images belong to the ABS group and no histopathological finding exists. D and D1 images belong to the Cd+ABS group. In the group given Cd+ABS, mononuclear cell infiltration is shown with a yellow arrow and hemorrhage in the vessel with a black arrow (A, B, C, and D X10) and (A1, B1, C1, and D1 X40). Statistical graphs of E Mononuclear cell infiltration, F hemorrhage, and congestion, G Thickening of alveoli.

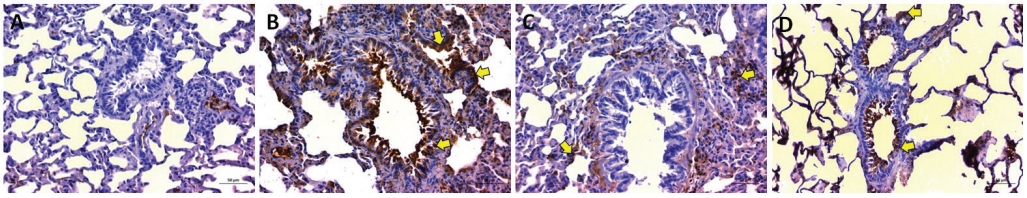

Figure 2;
Picture A of the control group is shown and there is no TNF-α synthesis. Cd group B picture is shown and TNF-α synthesis is marked with yellow arrows. The picture of the ABS group is indicated by C, the Cd+ABS group is indicated by D, and TNF-α syntheses are shown with yellow arrows (A, B, C, and D X20).

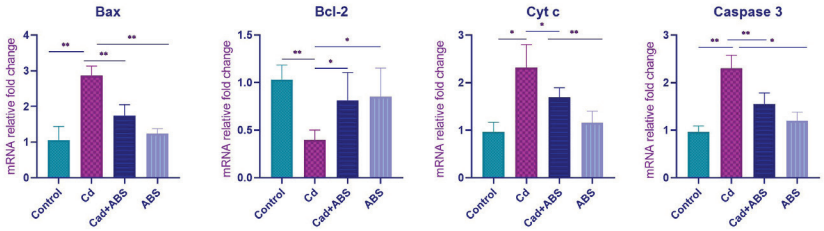

Figure 3;
mRNA relative fold change graph of genes in groups.
Cd: Cadmium, ABS: Ankaferd blood stopper, Bax: Bcl-2 associated X, Bcl-2: B-cell lymphoma 2, Cyt c: Cytochrome c. * $p<0.05$, ** $p<0.001$.

Table 2 Statistical analysis of TNF immunohistochemistry scoring.

	Groups				
	Control	**Cd**	**Cd+ABS**	**ABS**	**p-value**
TNF-α	0.00±0.00	2.50±0.53	1.37±0.52	0.50±0.43	a^{**}, b^{**}, $c^{**}d^{\#}$

Data were given as mean±SD (Standard deviation). The results of oxidative stress markers were assessed by a one-way ANOVA test. a Control vs. Cd., b Cd vs. Cd+ABS, c Cd vs. ABS, d Control vs. ABS. Cd: Cadmium, ABS: Ankaferd blood stopper, TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index. ** p<0.001, # p>0.05.

Table 3 Statistical analysis of TOS, TAS, and OSI values between groups in rat lung tissues

	Groups				
	Control	**Cd**	**Cd+ABS**	**ABS**	**p-value**
Parameters					
TOS	18.55±1.78	29.15±2.77	23.66±4.16	20.36±2.17	a^{**}, b^{*}, c^{**}, $d^{\#}$
TAS	1.63±0.24	0.78±0.26	1.25±0.11	1.33±0.27	a^{**}, b^{*}, c^{**}, $d^{\#}$
OSI	1.16±0.23	4.16±1.58	1.92±0.46	1.61±0.45	a^{**}, b^{**}, c^{**}, $d^{\#}$

Data were given as mean±SD (Standard deviation). The results of oxidative stress markers were assessed by a one-way ANOVA test. a Control vs. Cd., b Cd vs. Cd+ABS, c Cd vs. ABS, d Control vs. ABS. Cd: Cadmium, ABS: Ankaferd blood stopper, TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index, SD: Standard deviation. ** p<0.001, * p<0.05, # p>0.05.

Table 4 Relative mRNA expression levels and statistical analysis of genes in groups

	Groups				
	Control	**Cd**	**Cd+ABS**	**ABS**	**p-value**
Genes					
Bax	1.05±0.39	2.87±0.26	1.75±0.31	1.24±0.14	a^{**}, b^{**}, c^{**},$d^{\#}$
Bcl-2	1.03±0.15	0.39±0.10	0.81±0.29	0.85±0.30	a^{**}, b^{*}, c^{*}, $d^{\#}$
Cyt c	0.96±0.20	2.32±0.48	1.69±0.20	1.16±0.24	a^{**}, b^{*}, c^{**}, $d^{\#}$
Caspase 3	0.96±0.12	2.30±0.28	1.55±0.23	1.20±0.19	a^{**}, b^{**}, c^{**}, $d^{\#}$

Mean±SD levels of relative mRNA expression levels of genes were given and evaluated by a one-way ANOVA test. a Control vs. Cd., b Cd vs. Cd+ABS, c Cd vs. ABS, d Control vs. ABS. Cd: Cadmium, ABS: Ankaferd blood stopper, Bax: Bcl-2 associated X, BCL2: B-cell lymphoma 2, Cyt c: Cytochrome c. * p<0.05, ** p<0.001, # p>0.05.

Results of Oxidative Stress Markers

TOS and OSI values used to measure oxidative stress and TAS values indicating antioxidant levels were determined and compared between groups (Table 3). TOS and OSI levels increased in the Cd group compared to the control group, TAS levels were

significantly decreased (p<0.001). When the Cd and Cd+ABS groups were compared, TOS and OSI values decreased and TAS values increased significantly in the Cd+ABS group (p<0.004, p<0.001 and p=0.002).

Genetic Analysis Results in Rat Lung Tissues

Relative mRNA expression levels of pro-apoptotic and anti-apoptotic genes were determined in rat lung tissues in groups. When the Cd group was compared with the control group, a significant increase in the expression levels of Bax, Cyt c, and caspase 3 genes and a significant decrease in the expression levels of the Bcl-2 gene were determined in the Cd group (p<0.001). When the Cd group was compared with the Cd+ABS group, a significant decrease was observed in the Bax, cyt c, and caspase 3 gene expression levels in the Cd+ABS group, while there was a significant increase in the Bcl-2 gene (p<0.001, p=0.002, p<0.001 and p=0.007, respectively) (Table 4) (Figure 3).

Discussion

Cd causes significant toxic effects in many organs and tissues as a result of environmental and cigarette smoke factors. One of the organs where toxic effects are seen is the lung (30, 31). ABS consists of a blend of five plants and is used as a hemostatic agent in Turkish traditional medicine. ABS is also known to have antioxidant and anti-inflammatory effects (32). In this study, it was tried to determine the effect of ABS on the lung in rats with lung toxicity as a result of Cd exposure.

It has been shown that the most important mechanism of Cd toxicity in tissues and cells is the rise in ROS (8, 33). Cd achieves this action by attaching to the sulfhydryl groups of antioxidant enzymes and raising mitochondrial oxidative stress by disrupting the electron transport chain (9). In addition, oxidative stress activates mitochondrial apoptotic pathways and causes cell death (34). In a study conducted in 2018, it was stated that Cd decreased TAS levels and increased TOS levels in sublingual gland tissues (35). Miltonprabu et al. were reported that Cd causes inflammation and apoptosis by increasing mitochondrial oxidative stress (36). Kumar et al. In their study on the effect of Cd on lung epithelial cells, they determined that TNF-α and Bax levels increased, while Bcl-2 levels decreased (37). In a study by Yeşildağ et al. in 2021, it was revealed that Cd increased oxidative stress, inflammation, and apoptosis in lung tissues. It has been stated that Cd shows its oxidative effect through antioxidant and oxidant enzymes, its inflammatory effect by increasing TNF-α level, and its apoptotic effect by Bax, Caspase 3 increase, and Bcl-2 decrease (1). In our study, we found that Cd increases oxidative stress, inflammation, and apoptosis similar to the literature. We determined that Cd increased mitochondrial stress-related apoptosis as a result of Bax, Cyt c, cas 3 increase and Bcl-2 decrease, as well as increased oxidative stress. In addition, as a result of our histopathological evaluation, we determined that Cd causes pathological effects such as mononuclear cell infiltration in the lung, hemorrhage congestion, and thickening of the alveolar wall.

Each of the five plant extracts contained in ABS has its different properties and in the chemical analyzes, it has been determined that there are compounds with antioxidant properties (38). There is evidence that ABS regulates oxidative stress, inflammation, and apoptosis through these compounds. In the study of Buyuktiryaki et al. in 2019, it was determined that ABS decreased the oxidative stress markers TOS and OSI and increased the TAS level in necrotizing enterocolitis. It has also been stated that ABS reduces inflammation and apoptosis by affecting TNF-α and Caspase 3 levels. In addition, in the histopathological evaluation, fewer pathological findings were found in the ABS-treated group (24). When Kosmaz et al. investigated the negative effects of ABS in rats with experimental obstructive jaundice, they found that ABS decreased oxidative stress parameters and histopathologically, inflammation and fibrosis (32). Hasgül et al. investigated the effect of ABS on the damage caused by acetylsalicylic acid in the gastric mucosa and determined that ABS had a positive effect on tissue damage by reducing histopathological, inflammation, and oxidative stress (20). Hortu et al. were reported that it reduced TNF-α and apoptosis (25). Although previous studies have revealed that ABS has antioxidant, anti-apoptotic, and anti-inflammatory effects, no study has been found on the curative effect of Cd against the toxic effect of Cd in the lung. In our study, we determined that ABS application has an anti-inflammatory effect on lung toxicity induced by Cd and also has a healing effect by reducing oxidative stress and mitochondrial stress-related apoptosis. We determined that the healing effect of ABS on lung tissue was achieved by reducing hemorrhage and congestion, mononuclear cell infiltration, and alveolar wall thickness.

Our study has some limitations. The use of TUNEL method as well as gene expressions in the evaluation of mitochondrial apoptotic process may support the evidence on the effect of Cd and ABS on lung tissue. In addition, since this study is the first to evaluate

the effect of ABS on lung toxicity, we think that more studies should be done to evaluate its effectiveness.

Conclusion

As a result of our study, we determined that Cd causes histopathological changes by mononuclear cell infiltration, hemorrhage-congestion, and thickening of the alveolar wall in lung tissues. In addition, Cd increased oxidative stress, inflammation, and apoptosis. It positively affected the entire pathological process after treatment with ABS. For these reasons, we think that ABS can be used as an alternative for lung toxicities after Cd exposure.

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Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
The experimental protocol was approved by the local animal experiments ethics committee of Isparta Süleyman Demirel University with the number 15.09.2022/06-81.

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Availability of Data and Materials
All data generated during the study will be sent by the corresponding author upon reasonable request

Authors Contributions

M.Y.T: Planning of the study; Genetic analysis; Writing the Article.

P.S: Histopathological analysis, Immunohistochemical analysis, Editing of the article.

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