ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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PROTECTIVE ROLE OF ANKAFERD BLOOD STOPPER ON CADMIUM-INDUCED ACUTE NEPHROTOXICITY

ANKAFERD BLOOD STOPPER'IN KADMİYUMA BAĞLI GELİŞEN AKUT BÖBREK HASARINA ETKİSİ

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Öz

Amaç
Kadmiyum (Kd) insan sağlığı üzerinde zararlı etkilere neden olabilen çok toksik ve kanserojen bir ağır metaldir. Kd maruziyetine bağlı olarak özellikle böbreklerde toksisite gelişebilir. Ankaferd blood stopper (ABS), cerrahide hemostatik özelliği nedeniyle kullanılan bitkisel bir karışımdır. Ayrıca ABS'nin yara ve doku iyileşmesini artırdığı gösterilmiştir. Bu çalışmada, ABS'nin Kd kaynaklı böbrek hasarına karşı olası koruyucu etkilerini değerlendirmeyi amaçladık.

Gereç ve Yöntem
Otuz iki erkek sıçan rastgele 4 gruba ayrıldı: kontrol, Kd (kadmiyum klorür, 2,5 mg/kg tek doz ip), ABS (ABS, 1,5 ml/kg tek doz ip) ve Kd+ABS (kadmiyum klorür, 2,5 mg/kg tek doz ip-ABS, 1,5 ml/kg tek doz ip). Deney sonunda sıçanların serumlarından üre ve kreatinin seviyeleri ölçüldü. Ayrıca böbrek dokularından spektrofotometrik olarak total oksidan status (TOS), total antioksidan status (TAS) seviyeleri, süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GPx) enzim aktiviteleri ölçüldü. TOS ve TAS düzeylerinden oksidatif stres indeksi (OSI) hesaplandı. Ayrıca sıçanların böbrek dokusunda Bcl-2-associated X protein (Bax), B-cell-lymphoma-2 (Bcl-2), silenced information regulator 1 (SIRT1) ve p53'ün mRNA ekspresyonundaki değişiklikler qRT-PCR yöntemi ile değerlendirildi.

Bulgular
Kadmiyum grubunda serum üre, kreatinin düzeyleri ve doku oksidatif stres belirteçleri, TOS ve OSI değerleri kontrol grubuna göre anlamlı olarak yüksek ($p<0.05$), Gpx aktivitesi anlamlı olarak düşüktü ($p<0.05$). Ayrıca p53 ekspresyonu ve Bax/Bcl2 oranı Kd grubunda anlamlı artış göstermiştir ($p<0.05$). Ancak ABS tedavisi, Kd uygulanan grupta üre, kreatinin, TOS, OSI düzeylerini ve Bax/Bcl2 oranı ile p53 ekspresyonunu anlamlı düzeyde azaltmıştır ($p<0.05$).

Sonuç
Ankaferd blood stopper akut Kd maruziyetinde oksidatif stresi ve mitokondri aracılı apoptozu azaltarak koruyucu etkiler göstermiştir.

Anahtar Kelimeler: Kekik, Meyan, Oksidatif stress, Resveratrol, *Urtica dioica*

Abstract

Objective
Cadmium (Cd) is a very toxic and carcinogenic heavy metal that can cause harmful effects on human health. Toxicity may develop due to Cd exposure, especially in the kidneys. Ankaferd blood stopper (ABS) is a herbal mix that is used for its hemostatic properties in surgery. Also, ABS enhances wound and tissue healing. In this study, we aimed to evaluate the possible ameliorative effects of ABS in Cd-induced renal damage.

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Material and Method
Thirty-two male rats were randomly divided into 4 groups: control, Cd (cadmium chloride, 2.5 mg/kg single dose, ip), ABS (ABS, 1.5 ml/kg single dose ip), and Cd+ABS (cadmium chloride, 2.5 mg/kg single dose ip- ABS, 1.5 ml/kg single dose ip). At the end of the experiment, urea and creatinine levels were analyzed from the rats' serum. In addition, total oxidant status (TOS), total antioxidant status (TAS) levels, superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity were measured spectrophotometrically from renal tissues. The oxidative stress index (OSI) was calculated from TOS and TAS levels. Also, we evaluated alterations in the mRNA expression of Bcl-2-associated X protein (Bax), B-cell-lymphoma-2 (Bcl-2), silenced information regulator 1 (SIRT1), and p53 in kidney tissue of rats by using the qRT-PCR method.

Results
In the Cd group, serum urea, creatinine levels, and tissue oxidative stress markers, TOS and OSI were significantly higher while Gpx activity was significantly lower than in the control group ($p<0.05$). Also, the expression of p53 and in Bax/Bcl2 ratio significantly increased in the Cd group ($p<0.05$). But, ABS treatment significantly decreased urea, creatinine, TOS, OSI levels, and Bax/Bcl2 ratio, p53 expression in Cd applied group ($p<0.05$).

Conclusion
Ankaferd blood stopper showed protective effects by reducing oxidative stress and mitochondria-mediated apoptosis in acute Cd exposure.

Keywords: Licorice, Oxidative stress, Resveratrol, Thyme, *Urtica dioica*

Introductıon

Cadmium (Cd) is a divalent heavy metal element with atomic number 48. It is mostly used in nickel–cadmium batteries, steel plating, polyvinyl chloride (PVC), color pigments and alloys. In addition to industrial uses, Cd also exists in phosphate fertilizers due to manufacturing impurities (1). Human exposure to Cd is mainly caused by anthropogenic pollution. The main exposure route of Cd is the oral ingestion of contaminated foods and water. Besides, inhalation of contaminated air/cigarette smoking is another important exposure way (2).

Cd is a very toxic and carcinogenic element that has also a very long biological half-life (10 to 35 years). Although the intestinal absorption percent of the Cd is relatively low (%5-10), it has been stated that approximately half of the absorbed Cd accumulates in the kidneys (3). Therefore, kidneys are the major organs affected by Cd, and cumulative accumulation of Cd besides the acute exposure also underlies Cd toxicity. Nephrotoxicity mechanisms of Cd mainly depend on oxidative stress, and related consequences like the release of proinflammatory cytokines, loss of the regulation of autophagy, DNA damage, and finally apoptosis and cell necrosis (4-7).

Following intestinal absorption, Cd is transported to the liver and binds with the metallothionein proteins (Mt). The binding of the Cd to these proteins actually prevents toxic damage of Cd; however, Cd-bound proteins are released from the liver and transferred to the kidneys via blood circulation. After glomerular filtration and tubular reabsorption, Cd accumulates in the kidneys due to exceeding the Cd binding capacity of kidney Mt proteins, or dissociation of Cd from Mt proteins (5, 8, 9). Free Cd acts as a reactive oxygen species (ROS) stimulating agent. 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 (5). Increased ROS cause cellular damage and cell death through various mechanisms (10). Cadmium also causes genotoxicity through 1) DNA hypermethylation leading to reduced tumor suppressor gene expression and 2) histone acetylation causing increased proinflammatory cytokine levels such as IL-6, IL-1β, and TNF-α (4). Another cellular protein affected by Cd is silenced information regulator 1 (SIRT1). SIRT1 is a Class III histone deacetylase and it reveals protective and regulatory effects on oxidative stress, inflammation, apoptosis, autophagy, and cell survival (11). SIRT1 unleashes its effects through many target molecules including NF-κB and p53. SIRT1 deacetylates and inhibits NF-κB, which is a well-known inflammatory protein that is associated with cellular damage in many oxidative and inflammatory processes. Besides, SIRT1 also deacetylates and inhibits the p53 protein which plays a major role in apoptosis (12). Many studies have shown that oxidative stress causes a decrease in SIRT1 activity whereas upregulated expression of SIRT1 protects the kidneys or ameliorates the toxic effects caused by oxidative stress (7, 13-16). Bax and

Bcl2 are also well-known proteins that play significant roles in the development of oxidative stress and mitochondria-mediated apoptosis (17). Bax stands as a pro-apoptotic protein, on the contrary, Bcl2 exerts anti-apoptotic effects. So, the tissue Bax/Bcl2 ratio is commonly used to predict especially the severity of oxidative stress-related mitochondrial damage (18).

Antioxidant effects of herbals are well-known and are attracting the attention of researchers worldwide. Ankaferd Blood Stopper® (ABS) is a medical product used to control minor and major hemorrhages for its hemostatic properties. ABS contains the extracts of *Urtica diocia* (nettle) root, *Vitis vinifera* (grape) dried leaf, *Glycyrrhiza glabra* (licorice) dried leaf, *Alpinia officinarum* (galangal) dried leaf, and *Thymus vulgaris* (thyme) dried herb. In recent years, researchers have focused on the antioxidant, antimutagenic and anti-inflammatory effects of ABS as well as its hemostatic effects (19-22). Since oxidative stress is the key process in the development of Cd-induced nephrotoxicity, in our study we aimed to investigate the possible ameliorative/therapeutic effects of ABS on Cd toxicity.

Material and Method

Animals and Experiment Protocols

Our project was approved by the Suleyman Demirel University Animal Experiments Local Ethics Committee with the decision dated 15.09.2022 and numbered 06/76. Experiments were carried out in Suleyman Demirel University Animal Production and Experimental Research Center within the framework of the procedures determined by the relevant ethics committee.

32 male Wistar Albino rats were kept in 12 hours of light and 12 hours of a dark cycle at 22-24 degrees during the experiment and were fed ad libitum. The rats were divided into 4 groups, 8 animals in each cage.

On the day of the experiment, 1 ml of saline was administered intraperitoneally (ip) to the control (C) group from the right inguinal region.

The ABS group was administered 1.5 ml/kg ABS (Ankaferd Health Products Ltd., Türkiye) ip from the right inguinal region on the day of the experiment (23). The Cd group received cadmium chloride (CdCl2, Catalog no: 13667, Alfa Aeasar, USA) dissolved in saline at a dose of 2.5 mg/kg from the right inguinal region (24). ABS+Cd groups first received CdCl2 at a single dose of 2,5 mg/kg from the right inguinal region. One hour after the Cd administration, 1.5 ml/kg ABS was applied to the rats from the same area as ip.

One day after the Cd administration, rats were given 90 mg/kg Ketamine HCl and 10 mg/kg Xylazine for anesthesia. After sacrification, blood, and kidney tissue were taken for biochemical and genetic analysis. Kidney tissues were homogenized with Ultra Turrax Janke & Kunkel T-25 homogenizer (IKA® Werke, Germany) and Bandelin Hd 4200 Ultrasonic Homogenizer in phosphate buffer (pH: 7.4) to perform the biochemical analyzes. Then, the homogenates were centrifuged at 10000 rpm for 10 minutes and the supernatants were aliquoted. Serum was obtained from blood samples after centrifugation at 3000 rpm for 10 minutes and aliquoted. All aliquots were stored at -80°C until analyze day.

Biochemical Analyzes

Serum urea and creatinine levels were determined by spectrophotometric method with a Beckman Coulter AU5800 (Beckman Coulter, USA) and a commercial kit compatible with this device to show the kidney functions. To evaluate the oxidant-antioxidant status in kidney tissues of rats, TAS and TOS levels were measured spectrophotometrically using commercial kits (Rel Assay Diagnostics, Türkiye) by Beckman Coulter AU5800 autoanalyzer (Beckman Coulter, USA). The formula (OSI = [(TOS / (TAS) × 100]) was used to calculate the OSI values (25-27).

Superoxide dismutase (SOD) activity was measured from kidney tissue supernatants by the xanthine oxidase method using the Ransod commercial kit (Randox Laboratories, United Kingdom). GPx activity was measured by the Valentine and Paglia method using a Ransel commercial kit (Randox Laboratories, UK). Protein levels of supernatants were determined spectrophotometrically with a Beckman Coulter AU5800 autoanalyzer and compatible protein assay kit (Beckman Coulter, USA).

Genetic Analyzes

RNA isolation

RNA isolation of kidney samples was performed with the GeneAll Ribospin RNA isolation kit (GeneAll Biotechnology, Korea). The amount and purity of the obtained RNAs were measured with the nanodrop spectrophotometer (Thermo Scientific NanoDrop™, USA). The concentration of each isolated RNA sample was standardized at 500ng/µl and stored at -80°C for the cDNA synthesis.

cDNA Synthesis

cDNA synthesis was carried out in a thermal cycler

according to the protocol of A.B.T. cDNA Synthesis Kit (Atlas Biotechnology, Türkiye). The following concentrations were prepared 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 determined using the NCBI database. The genes and specific primer sequences used in the expression step are given in Table 1. Expression levels were measured by the Biorad CFX96 (Bio-Rad Laboratories, USA) real-time qPCR instrument using the A.B.T. 2X qPCR SYBR-Green MasterMix (Atlas Biotechnology, Türkiye). 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. The normalized data were made available for statistical analysis using the ΔΔCt method.

Statistical Analyzes

Data distributions were analyzed using Shapiro-Wilk's test. Since our data were normally distributed, a one-way analysis of variance (ANOVA) test was used to compare the mean differences between groups. The homogeneities of the data variances were evaluated with the Levene homogeneity test. Post-hoc Tukey test was used to evaluate inter-group comparisons. A p-value of less than 0.05 was considered significant.

Results

Oxidative Stress Markers

Tissue TAS and SOD levels were not significantly different between groups (TAS: $F(3,28)=2.673$, $p=0.067$; SOD: $F(3,28)=2.641$, $p=0.069$). However, there were significant differences in TOS, OSI, and GPx levels between groups (TOS: $F(3,28)=21.08$, $p<0.001$; OSI: $F(3,28)= 46.13$, $p<0.001$; GPx: $F(3,28)=8.367$, $p<0.001$; Table 2).

TOS levels were significantly elevated in the Cd group compared to the other groups ($p<0.001$ for all). OSI levels were also significantly increased in the Cd group than in the other groups (Cd vs C and Cd vs ABS, $p<0.001$; Cd vs Cd+ABS, $p=0.005$). There was also a significant increase in OSI levels in the Cd+ABS group compared to the both C and ABS groups ($p<0.001$ for both, Table 2).

GPx levels were significantly decreased in the Cd group compared to the both C and ABS groups (Cd vs C, $p<0.001$; Cd vs ABS, $p=0.019$).

Kidney Functions

One-way ANOVA showed significant differences in both urea and creatinine levels between groups (Urea:

Table 1 Specific primers used in PCR analysis

Gene	Specific Primer Sequence	Product length
GAPDH (HouseKeeping)	F: AGGTTGTCTCCTGTGACTTC	130 bp
	R: CTGTTGCTGTAGCCATATTC	
SIRT1	F: GGTAGTTCCTCGGTGTCCT	152 bp
	R: ACCCAATAACAATGAGGAGGTC	
P53	F: GAGTGCTGAAGGAGATCAATGAG	145 bp
	R: GTGGTCAGTCCGAGCCTTTT	
Bcl-2	F: ATCGCTCTGTGGATGACTGAGTAC	134 bp
	R: AGAGACAGCCAGGAGAAATCAAA	
Bax	F: AGGGTGGCTGGGAAGGC	93 bp
	R: TGAGCGAGGCGGTGAGG	

F: Forward, R: Reverse, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, SIRT1: Silenced information regulator 1, Bcl-2: B-cell lymphoma 2, Bax: Bcl-2-associated X protein

Table 2 Results of tissue oxidative stress parameters and serum kidney function tests

	Control Mean±SD	Cadmium Mean±SD	ABS Mean±SD	Cadmium+ABS Mean±SD
TAS (mmol Trolox Eq/g protein)	0.18 ± 0.03	0.20 ± 0.05	0.18 ± 0.02	0.16 ± 0.02
TOS (µmol H_2O_2/g protein)	1.62 ± 0.39	3.53 ± 0.82[a,b,c]	1.66 ± 0.43	2.26 ± 0.45
OSI (Arbitrary Unit)	0.90 ± 0.15	1.79 ± 0.15[a,b,d]	0.90 ± 0.23	1.45 ± 0.19[e,f]
SOD (U/mg protein)	15.57 ± 2.97	12.11 ± 2.32	14.19 ± 1.99	12.81 ± 3.21
GPx (U/mg protein)	2.49 ± 0.52	1.41 ± 0.34[a,g]	2.10 ± 0.38	1.95 ± 0.49
Urea (mg/dl)	41.45 ± 11.08	75.93 ± 14.17[a,b,c]	36.28 ± 13.21	44.67 ± 6.17
Creatinine (mg/dl)	0.36 ± 0.13	0.81 ± 0.31[a,b,d]	0.31 ± 0.04	0.42 ± 0.15

'a': Cadmium vs. Control, p<0.001; 'b': Cadmium vs. ABS, p<0.001; 'c': Cadmium vs. Cadmium+ABS, p<0.001;
'd': Cadmium vs. Cadmium+ABS, p<0.01; 'e': Control vs. Cadmium+ABS, p<.001; 'f': ABS vs. Cadmium+ABS, p<0.01;
'g': Cadmium vs. ABS, p<0.05.

F(3,28)=19.13, p<0.001; Creatinine: (F(3,28)=12.06, p<0.001). Serum urea levels were found to be significantly elevated in the Cd group compared to the other groups (p<0.001, Table 2.) Creatinine levels were also found significantly high in the Cd group compared to the other groups (Cd vs C and Cd vs ABS, p<0.001; Cd vs Cd+ABS, p=0.001). Serum urea and creatinine levels were not significantly different in the ABS group compared to the C group.

Relative mRNA expressions

Kidney p53 mRNA expressions were elevated 2.11 fold in the Cd group compared to the C group (p<0.001, Fig. 1). ABS treatment with Cd administration decreased the p53 mRNA expression 0.55 fold compared to the Cd-only treated group (p=0.004, Fig. 1).

Mean SIRT1 mRNA expression was also decreased -but not significant- in the Cd group 0.59 fold compared to the C group (p=0.116, Fig. 1). ABS treatment with Cd caused a non-significant 1.61 fold increase in SIRT1 expression compared to the Cd group (p=0.201, Fig. 1). Also, SIRT1 expression was significantly 1.85 fold higher in the ABS group compared to the Cd group (p=0.038, Fig. 1).

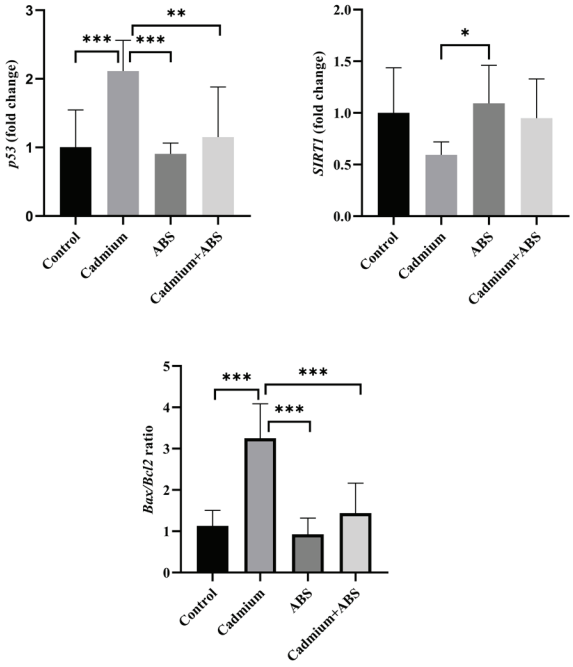

Figure 1:
Figure 1. Fold changes in mRNA expressions of p53 and SIRT1 genes and expression ratio of Bax to Bcl2 gene.
'***': p<0.001; '**': p<0.01; '*': p<0.05.

There was a significant increase in Bax/Bcl2 ratio in the Cd group compared to the other groups ($p<0.001$ for all, Fig. 1). The ratio was 2.88 fold elevated in the Cd group compared to the C group and decreased 0.44 fold in the Cd+ABS group compared to the Cd group (Fig. 1).

Discussion

In the current study, we have shown the negative effects of the ip. Cadmium administration on kidney functions via elevated serum urea and creatinine levels. For the underlying mechanisms, we have focused on oxidative stress markers and related genetic changes, as mentioned in the literature data. We have confirmed the increased oxidative stress by measuring tissue TAS, TOS, OSI levels, and activities of SOD and GPx. We also showed the changes in mRNA expressions of p53, SIRT1, Bax, and Bcl2, which are some of the prominent oxidative stress-affected proteins. As we aimed in the study, we showed that ABS - a herbal mix medical product- ameliorated the Cd-induced nephrotoxicity in rats.

The antioxidant effects of the herbals are well-known, and the intracellular molecular effects of many of them are well documented in the literature. The most important compounds in herbal products that act as antioxidants are polyphenols, sterols, carotenoids, and flavonoids. These compounds have free radical-scavenging effects mainly by donating a hydrogen atom to the free radicals or quenching oxygen, and some of them chelate metal ions that are taking part in oxidation reactions (28). Specifically for this study, all of the plant extracts in the ABS are known to have strong antioxidant and chelating properties.

There are a limited number of studies evaluating the protective effects of *Urtica dioica* in heavy metal toxicity. Siouda et al. showed the renal protective effects of *Urtica dioica* against HgCl2 administration by measuring serum urea, creatinine levels, and kidney glutathione (GSH) levels, (29). Similarly, in 2021, Aldulaimi et al. showed that *Urtica dioica* treatment against copper sulfate toxicity caused a decrease in high serum urea and creatinine levels (30). Although these studies are not sufficiently detailed, they may be considered as remarkable as they reflect the renoprotective effects of *Urtica dioica* in heavy metal poisoning.

Resveratrol is the most important polyphenolic component of *Vitis vinifera* leaf, which is known to be responsible for its antioxidant and protective effects (31). Recent studies have shown strong ameliorative effects of resveratrol in heavy metal-induced toxicity (32, 33). Among these studies, the most similar and comparable to our study was conducted by Cirmi et al. in 2021. In their study, the combined and separate effects of bergamot juice, curcumin, and resveratrol in Cd-induced kidney damage were evaluated. 2 mg/kg $CdCl_2$ was administered ip for 14 days to mice, concurrently with and without 20 mg/kg oral resveratrol. It was shown that Cd injection caused significant deteriorative alterations in the levels of urea, creatinine, GSH, GPx, and mRNA expressions of p53, Bax, and Bcl2. Resveratrol treatment has been found to reduce serum urea and creatinine levels and significantly increase kidney GSH and GPx levels. It was also found that resveratrol treatment reduced p53 and Bax mRNA expressions, but didn't change Bcl2 expressions. Unlike ours, neither the Bax/Bcl2 ratio was reported nor SIRT1 was studied in this study. In addition, this work has confirmed the oxidative stress with GSH and GPx, but we further measured TAS, TOS, OSI, and SOD levels. Even though the treatment durations and doses were different from our study, the results were strongly concordant with ours (33).

Dirican and Turkez found that Glycyrrhiza glabra extract ameliorated the genotoxicity, cytotoxicity, and oxidative stress (34). Mohamed et al. found that Glycyrrhiza glabra extract increased kidney GSH, SOD, catalase (CAT), kidney injury molecule (KIM 1) levels and reduced serum urea, creatinine levels, and kidney thiobarbituric acid reactive substances (TBARS) levels in rats treated with 10 mg/kg CdCl2 for 4 weeks (35). In addition to these studies, a study performed on fish (*O. niloticus*) indicated that *Glycyrrhiza glabra* reduced heavy metal accumulation in the flesh of the fish (36).

Arab et al. comprehensively studied the effects of *Alpinia officinarum* on Cd-induced renal damage. They reported that *Alpinia officinarum* extract improved kidney functions, mitigated oxidative stress, and showed antiapoptotic and cytoprotective effects throughout Bax/Bcl2 and SIRT1-mediated pathways (14).

Thymus vulgaris, another component of the ABS, has also been indicated to relieve renal functions and oxidative stress in Cd-induced nephrotoxicity (37, 38). The results we found in our research are generally compatible with the studies mentioned above, but these studies separately investigate the effects of the components in ABS on heavy metal toxicity. There is no research that examined the effects of all components of ABS together on heavy metal toxicity. Studies on ABS in the literature generally investigate its usage in

surgery. Nowadays, investigating the effects of ABS at the molecular level is gaining importance.

Recent studies suggest that ABS induces apoptosis and exhibits antineoplastic properties in osteosarcoma, malignant melanoma, colon cancer, and bladder cancer cells (39). But in case of severe damage in normal cells, ABS may show cytoprotective effects. For example, Koşmaz and Durhan suggested that ABS could mitigate experimental liver damage via its anti-inflammatory and antioxidant properties (40). Buyuktiryaki et al. found that ABS ameliorates necrotizing enterocolitis by suppressing apoptotic factors (41). Huri et al performed partial nephrectomy in rats and when the bleeding is mild or moderate, ABS induced renal tubular apoptosis; however, in the case of massive bleeding from the kidney tissue due to surgery, ABS decreased apoptosis in renal cells (42).

The main mechanism accused in the pathogenesis of Cd-induced toxicity is oxidative stress. Although TAS and SOD levels remained unchanged, the Cd group showed increased TOS and OSI levels and decreased GPx levels in our study. Since we have observed the acute effects of Cd and ABS, alterations in TAS and SOD may not have emerged yet. Although, our genetic analysis results suggested that ABS has a prominent anti-apoptotic effect on Cd-induced nephrotoxicity via Bax/Bcl2 pathway whereas this effect seems to be independent of antioxidant activity. Also, the change in SIRT1 levels was not statistically significant but the alterations in p53 levels support that ABS reduced apoptosis in Cd-induced kidney damage. SIRT1 also modulates inflammatory pathways, and ABS may have reduced apoptosis by exerting anti-inflammatory effects. However, as a limitation, inflammatory pathways were not investigated in our study.

Conclusion

Taken together, we have revealed that ABS showed renoprotective effects by decreasing oxidative stress and mitochondria-mediated apoptosis, even in acute Cd exposure. In further studies, the intracellular effects of ABS may be elucidated in more detail by histological analyzes and measurement of protein levels. In addition, the application of different treatment doses and durations of ABS can be useful in terms of the possibility of seeing beneficial changes depending on the dose and time.

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

Ethical Approval
This study was approved by the Suleyman Demirel University Animal Experiments Local Ethics Committee with the decision dated 15.09.2022 and numbered 06/76. Experiments were carried out in Suleyman Demirel University Animal Production and Experimental Research Center within the framework of the procedures determined by the relevant ethics committee.

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Availability of Data and Materials
Data is available on request from the authors.

Authors Contributions
II: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing-original draft.

HIB: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Writing-review & editing.

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