Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2026;51(1):272-281
DOI: 10.17826/cumj.1784518

RESEARCH

Urtica dioica L. attenuates bisphenol A-induced hepatotoxicity in rats by suppressing apoptosis and inflammation

Urtica dioica L., sıçanlarda apopitoz ve inflamasyonu baskılayarak bisfenol A kaynaklı hepatotoksisiteyi hafifletir

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Abstract

Purpose: The aim of this study was to investigate the protective effect of Urtica dioica L. (UD) against liver damage induced by bisphenol A (BPA) in rats.
Materials and Methods: To this end, 48 healthy male Wistar albino rats were randomly divided into six groups: control (0.5 ml of normal saline); BPA (50 mg/kg); UD100 (100 mg/kg); UD200 (200 mg/kg); BPA+UD100; and BPA+UD200. All administrations were carried out for 56 days. The effects of the BPA and UD administrations on expression levels of Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2) and tumor necrosis factor-α (TNF-α) in liver tissue samples were evaluated using Western blotting and immunohistochemical analyses.
Results: BPA caused significant increases in the Bax/Bcl-2 expression ratio (+45.2-fold) and TNF-α levels (+12.2-fold) in liver tissue. In groups treated with UD at 100 and 200 mg/kg doses, apoptosis was suppressed by upregulating Bcl-2 expression and downregulating Bax expression. Furthermore, the co-administration of UD was observed to significantly reduce the histopathological damage caused by BPA in liver tissue. In the BPA+UD100 group, inflammation was suppressed by downregulating TNF-α expression (-18.8-fold); however, in the BPA+UD200 group, TNF-α expression levels were higher than in the control group.
Conclusion: These findings suggest that UD treatment can mitigate the adverse effects of BPA on liver tissue and enhance tissue healing in rats.

Keywords: Bisphenol A, urtica dioica L., hepatotoxicity, apoptosis, inflammation

Öz

Amaç: Bu çalışmanın amacı, sıçanlarda bisfenol A (BPA) ile indüklenen karaciğer hasarına karşı Urtica dioica L. (UD)'nin koruyucu etkisini araştırmaktır.
Gereç ve Yöntem: Bu amaçla, 48 sağlıklı erkek Wistar albino sıçan rastgele altı gruba ayrıldı: kontrol (0.5 ml normal salin); BPA (50 mg/kg); UD100 (100 mg/kg); UD200 (200 mg/kg); BPA+UD100 ve BPA+UD200. Tüm uygulamalar 56 gün boyunca gerçekleştirildi. BPA ve UD uygulamalarının karaciğer doku örneklerinde Bcl-2 ilişkili X protein (Bax), B-hücreli lenfoma 2 (Bcl-2) ve tümör nekroz faktör-α (TNF-α) ekspresyon düzeyleri üzerine etkileri, Western blot ve immunohistokimyasal analizler kullanılarak değerlendirildi.
Bulgular: BPA, karaciğer dokusunda, Bax/Bcl-2 ekspresyon oranı (+45.2 kat) ve TNF-α düzeylerinde (+12.2 kat) anlamlı artışlara neden oldu. 100 ve 200 mg/kg dozlarında UD ile tedavi edilen gruplarda Bcl-2 ekspresyonu yukarı düzenlenerek ve Bax ekspresyonu aşağı düzenlenerek apopitoz baskılandı. Ayrıca, UD'nin eşzamanlı uygulamasının, karaciğer dokusunda BPA'nın neden olduğu histopatolojik hasarı önemli ölçüde azalttığı gözlendi. BPA+UD100 grubunda, TNF-α ekspresyonu aşağı düzenlenerek (-18.8 kat) inflamasyon baskılandı; ancak BPA+UD200 grubunda, TNF-α ekspresyon seviyeleri kontrol grubundan daha yüksekti.
Sonuç: Bu bulgular, UD tedavisinin BPA'nın karaciğer dokusu üzerindeki olumsuz etkilerini hafifletebileceğini ve sıçanlarda doku iyileşmesini artırabileceğini göstermektedir.

Anahtar kelimeler: Bisfenol A, urtica dioica L., hepatotoksisite, apopitoz, inflamasyon

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Received: 15.09.2025 Accepted: 23.02.2026

INTRODUCTION

Bisphenol A (BPA), formed through the condensation of two phenol molecules and one acetone molecule in the presence of concentrated sulfuric acid, is generally used in the production of polycarbonates, epoxy resins, food storage containers, dental fillings, mineral water bottles, and baby bottles. BPA is utilized as a thin layer to protect food and prevent direct contact between food and metal cans. BPA is considered a biodegradable compound[1]. It has been reported that BPA is detected in varying concentrations in urine, blood, placenta, amniotic fluid, umbilical cord, and breast milk[2]. Due to its structural similarity to the estrogen hormone, BPA can mimic the effects of endogenous estrogen by disrupting the homeostasis of endocrine systems[3]. Consequently, it plays a pathogenic role in certain endocrine disorders, such as precocious puberty, polycystic ovary syndrome (PCOS), and infertility. Additionally, it has been reported to have adverse effects on the nervous system, cardiovascular disorders, immune system, diabetes, and cancer in humans[1]. It has also been suggested that BPA interferes with signaling pathways related to androgen receptors, thyroid hormone receptors, and other hormones[4].

The liver, which is critically important in controlling many physiological processes in the body, is the primary organ responsible for the detoxification of various drugs and xenobiotics. Consequently, it plays a significant role in the metabolism and elimination of ingested BPA[5]. It is known that BPA can increase liver enzymes, inflammation, oxidative stress, lipid peroxidation, and insulin resistance, as well as induce the progression of hepatic tumors[6].

Additionally, it has been reported that BPA is associated with alterations in the blood-lipid profile, interferes with the oxidant/antioxidant mechanism in the liver[5], and causes the formation of multinucleated giant cells in hepatocytes[7]. Studies have also indicated that BPA induces histopathological changes in liver tissue, such as sinusoidal congestion, cytoplasmic vacuolization, and leukocyte infiltration[8].

Apoptosis, which eliminates abnormal cells to support homeostasis and tissue development, is known as programmed cell death. Dysregulation of this process, which is controlled by various genes, can be induced by multiple factors, particularly chemical toxicants[2]. It has been shown that hepatotoxic BPA exacerbates the inflammatory response by increasing the levels of inflammation-related cytokines in liver tissue and causes a significant increase in histopathological changes by triggering apoptosis in liver cells[5,9]. Tumor necrosis factor-α (TNF-α), which is a pleiotropic cytokine involved in tissue healing, plays a role in the induction of apoptosis in liver cells through the activation of the Bcl-2-associated X protein (Bax)[5,10]. TNF-α, whose hepatic expression increases as part of the inflammatory response following BPA exposure, also causes damage to liver tissue[5].

The stinging nettle (*Urtica dioica* L.) (UD), belonging to the Urticaceae family, is an annual or perennial herbaceous plant with stinging hairs. UD is a highly nutritious food rich in bioactive compounds (phenolic acids and flavonoids), chemical components (calcium, copper, and iron), and vitamins (A, B, C, E, and K)[11].

UD is known to possess numerous pharmacological activities, including antioxidant, immunomodulatory, antiviral, antibacterial, analgesic, antihypertensive, anti-inflammatory, antidiabetic, antihyperlipidemic, anticarcinogenic, hepatoprotective, and radioprotective effects[12]. Additionally, it is known to alleviate cold symptoms, rheumatic pains, stomach aches, and cough[13] and contribute to lowering blood pressure by reducing vascular tension[14]. It has been reported that UD reduces lipid peroxidation and liver enzyme activity[15] and exerts a protective effect by increasing arylesterase, paraoxonase, and catalase activity in liver tissues[16].

Considering the specified pharmacological properties of UD, more details are required to determine its effectiveness against BPA-induced liver damage. To the best of our knowledge, there is no published report demonstrating the effect of UD against BPA-induced liver toxicity in rats. Therefore, in this study, it was aimed to demonstrate the protective efficacy of UD application against BPA-induced liver damage by modulating apoptosis and inflammation against tissue damage through histopathological immunohistochemical and molecular analyses. Furthermore, it is considered that a deeper understanding of UD's protective role in this study could enable the development of new treatment options against liver damage associated with BPA or different xenobiotics thereby making a significant contribution to the literature.

MATERIALS AND METHODS

Preparation of UD extract

The fresh aerial parts of UD were collected from Hatay province in Türkiye. The plant samples were identified and recorded in Herbarium of Centre for Implementation and Research of Plant Health Clinic of Hatay Mustafa Kemal University by Prof. Dr. Ilhan Uremis. The voucher specimen number was (MKUBK-H-0296). The plant was first washed with distilled water, dried in an air stream, and ground into powder using a blender. The powdered plant material was extracted in a 70% ethanol-distilled water mixture for 72 h. Then, the mixture was filtered using Whatman No. 44 (Macherey-Nagel, Düren, Germany), and the solvent was removed using a rotary evaporator (IKA RV 3 V Flex, IKA, Staufen, Germany). The remained was lyophilized and stored at -20°C until use[17].

Animals and experimental design

The 48 adult male Wistar Albino rats used in the study (aged 2 months, approximately weighing 200 g) were obtained from the Experimental Research and Application Centre of Hatay Mustafa Kemal University. Animal care and all experimental procedures were conducted by the research team in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Ethical approval was obtained from the Local Ethics Committee of Animal Experiments at Hatay Mustafa Kemal University for the conduct of the study (Approval Date: 24/06/2025, Approval Number: 2025/05-07). The rats were housed in standard cages and kept in a temperature-controlled room (24±3°C) under a 12-h light/dark cycle. The rats were provided with water and standard chow *ad libitum*. The rats were randomly divided into six groups (eight rats in each group):

Group I (Control group): 0.5 ml normal saline;

Group II (BPA group): 50 mg/kg of BPA (CAS No: 80-05-7, Sigma-Aldrich, USA)[18];

Group III (UD100 group): 100 mg/kg of UD extract;

Group IV (UD200 group): 200 mg/kg of UD extract;

Group V (BPA+UD100 group): 100 mg/kg UD extract treatment 30 min before each 50 mg/kg BPA administration;

Group VI (BPA+UD200 group): 200 mg/kg UD extract treatment 30 min before each 50 mg/kg BPA administration. BPA and UD extract were dissolved in normal saline. All administrations were carried out daily by oral gavage for 56 days. The UD treatment doses were chosen based on the results of previously published studies[19,20].

The rats in the control and experimental groups were weighed 24 h after the last dose and then decapitated under 10 mg/kg xylazine hydrochloride (Rompun 2%, Bayer, Leverkusen, Germany) and 100 mg/kg ketamine hydrochloride (Keta-Control, Doğa İlaç, Istanbul, Türkiye) anesthesia. The liver tissues were excised and weighed. One of the tissues was stored in a freezer at -80°C for molecular analysis, and the rest were fixed in 10% formalin solution for histopathological and immunohistochemical analysis.

Histopathological analysis

The collected liver tissues were fixed in 10% formalin solution. They were then dehydrated using a series of graded ethanol concentrations. Subsequently, they were treated with xylene (CAT. No: 108297, Merck, Germany) and embedded in paraffin. Sections of 5 μm thickness were obtained from the tissues using a microtome (Leica RM2245, Leica Biosystems, Heidelberg, Germany), and hematoxylin and eosin staining was performed. The sections were examined under a microscope (Zeiss Imager A2, Oberkochen, Germany) for histopathological evaluation[14].

Immunohistochemical analysis

Liver tissue sections, 5 μm thick, were incubated at 60°C for 6 h. Subsequently, they were passed through xylene and a descending alcohol series. The sections were placed in distilled water and washed with phosphate buffered saline (PBS) (CAT. No: P4417, pH 7.2, Sigma-Aldrich, USA). The sections were heated in an ethylenediaminetetraacetic acid (EDTA) solution (CAT. No: ab93680, pH 8.0, Abcam, USA) using a microwave oven for 3x6 min and then antigen retrieval was performed. The sections were washed with PBS and incubated in a 3% hydrogen peroxide (H_2O_2) solution (CAS No: 7722-84-1, ThermoFisher, USA) for 20 min. After this period, they were washed with PBS and blocked with Ultra V Block solution (CAT. No: TA-125-UB, ThermoFisher, USA). Primary antibodies (Bax, CAT. No: sc-20067; Bcl-2, CAT. No: sc-7382; TNF-α, CAT. No: sc-52746, Santa Cruz Biotechnologies, USA) were applied to the tissue samples in the sections and incubated

overnight at 4°C. The sections were washed with PBS, and a secondary antibody was applied for 14 min. The samples were then washed again with PBS and streptavidin-peroxidase (CAT. No: TS-015-HR, Thermo Scientific, USA) was applied for 15 min. It was dropped with 3,3'-diaminobenzidine (DAB) to the samples (CAS No: 91-95-2, Sigma-Aldrich, USA), incubated for 1-2 min, and washed with PBS. The sections were incubated in Harris hematoxylin and washed under tap water. The sections were then passed through an ascending alcohol series and placed in xylene. The sections were covered with entellan (CAT. No: 107960, Merck, Germany) and subsequently visualised under a Zeiss Imager A2 photomicroscope[12].

Western blot analysis

The analysis of Bax, B-cell lymphoma 2 (Bcl-2), and TNF-α was performed using Western blotting, with some modifications to the method described by Shi et al[3]. The liver tissues stored at -80°C were crushed in liquid nitrogen. Then, radioimmunoprecipitation assay (RIPA) buffer (CAT. No: 89900, Thermo Scientific, USA), protease-phosphatase inhibitor cocktail (CAT. No: 78440, Thermo Scientific, USA), and benzonase nuclease (CAT. No: 9025-65-4, Sigma-Aldrich, USA) were added to the samples to release the proteins. The total protein amount in each lysate was determined using the bicinchoninic acid (BCA) protein assay kit (CAT. No: 23225, ThermoFisher, USA). Equal amounts of proteins (20 µg) were loaded onto a 10% TGX stain-free fastcast acrylamide gel (CAT. No: 1610183, Bio-Rad, USA) and separated by electrophoresis. Subsequently, the proteins were transferred to a polyvinylidene fluoride (PVDF) transfer membrane (CAT. No: 12023927, Bio-Rad, USA).

The membranes were blocked with 5% non-fat milk powder (CAT. No: SKI400, BioShop Canada Inc., Canada) for 1 h at room temperature and incubated overnight at 4°C with primary antibodies (Bax, Bcl-2, TNF-α, and anti-GAPDH; CAT. No: AF7021, Affinity Biosciences, USA). The membranes were washed with PBS-T (PBS-Tween) and incubated with a secondary antibody (CAT. No: 31430, HRP-goat anti-mouse, ThermoFisher, USA) for 1 h at room temperature. The protein bands were visualised using ECL (CAT. No: BMU102-EN, Abbkine Scientific Co., USA) with ChemiDoc MP. Band intensities were analyzed using the Image Lab 6.1 software.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 10 software (GraphPad Software, San Diego, CA, USA). Data are expressed as mean±standard deviation (SD). To check the normality of the data distribution, the Shapiro–Wilk test was used. The homogeneity of the variance was evaluated by the using Levene test. A value of $p>0.05$ was accepted to indicate a normal distribution and the homogeneity of the variance. Data with normal distribution among multiple groups were analyzed using one-way ANOVA, and post hoc analysis was conducted using Tukey HSD test. Power analysis and sample size determination for the current study were performed using the statistical software G*Power (version 3.1.9.7). The analysis showed that a total of 48 rats would be sufficient, assuming an effect size of 0.80, statistical power of 0.86, and an alpha level of 0.05. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Evaluation of liver weight measurements

Figure 1 shows the absolute and relative liver weights of rats in the control and experimental groups. No statistically significant differences were found in absolute or relative liver weight values when the groups were compared.

Histopathological results

In the liver tissue sections of the control group, the hepatocyte cords were arranged in a radial pattern with clearly visible basal membranes. The sinusoidal structures were regular, the epithelial cell nuclei were flattened and the hepatocytes and their nuclei appeared rounded. The boundaries of the central vein were distinct and blood cells were observed in the lumen (Figure 2A). In the BPA group, disruptions to the radial structure of the hepatocyte cords were noted. Some hepatocytes adjacent to sinusoids exhibited shape deformities and loss of nuclei. The lumen of the central vein was irregular and, in some hepatocytes, the boundaries were indistinct and the nuclear structures were impaired (Figure 2B). In the UD100 group, the radial arrangement of the hepatocyte cords was regular; however, the hepatocyte boundaries were indistinct. The epithelial boundaries of the widened sinusoidal lumens and some hepatocyte nuclei were indistinct. The central

vein lumen was regular with distinct boundaries (Figure 2C). In the UD200 group, the radial arrangement of hepatocyte cords was irregular; however, the hepatocyte boundaries and nuclei were distinct. Sinusoids appeared narrower and more regular than in the UD100 group. The central vein lumen was regular, with prominent blood cells (Figure 2D). In liver sections of the BPA+UD100 group, the radial arrangement of hepatocyte cords appeared more regular than in the BPA group. The nuclei of sinusoidal epithelial cells were prominent, flattened and regular, while the hepatocyte boundaries were more distinct and their nuclei were round. The central vein lumen was regular, but the blood cells were not distinct (Figure 2E). Compared to the BPA group, the radial arrangement of hepatocyte cords in liver sections of the BPA+UD200 group was much more regular, with hepatocytes and their nuclei appearing distinct and regular. The sinusoids were parallel to the remark cords, with distinct boundaries. The lumen of the central vein appeared regular and the nuclei of the epithelial cells were clearly defined (Figure 2F).

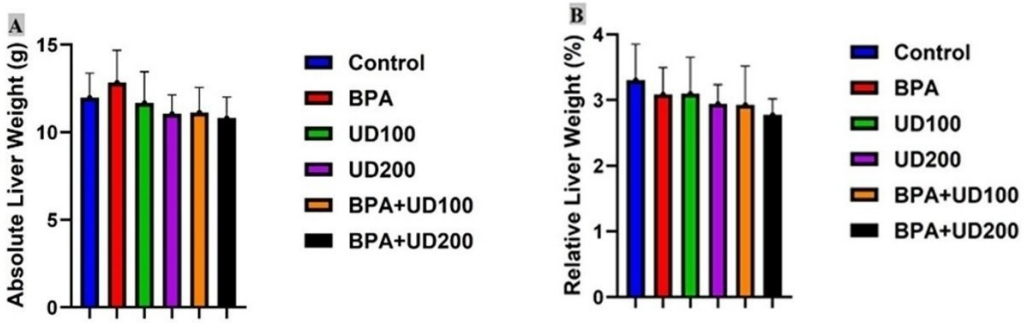


Figure 1. Effects of BPA and UD on the absolute and relative liver weights.
(Bars represent the mean±SD of the experiments. BPA: Bisphenol A; UD: Urtica dioica).

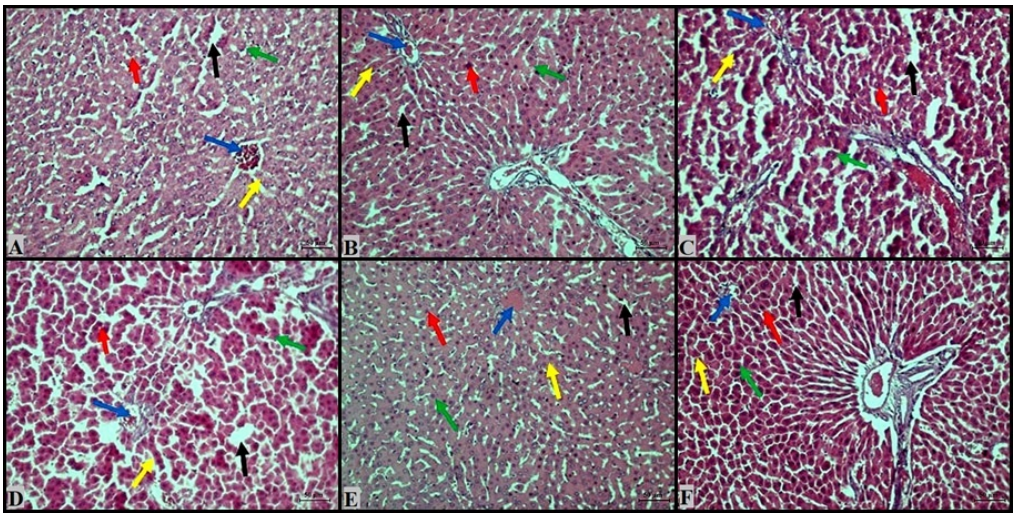


Figure 2. Hematoxylin-eosin staining of liver sections. Liver tissue sections of the control (A), BPA (B), UD100 (C), UD200 (D), BPA+UD100 (E), and BPA+UD200 (F) groups. Red arrow: Hepatocyte nucleus, yellow arrow: Remarkable cord, blue arrow: Portal vein, green arrow: Hepatocyte, black arrow: Sinusoid. (Scale bar: 50 μm, Hematoxylin & Eosin staining, magnification: 20x). (BPA: Bisphenol A; UD: Urtica dioica).

Immunohistochemical results

Figure 3 shows the Bax, Bcl-2 and TNF-α immunostaining of liver tissues from the control and experimental groups. In liver sections from the control group, Bax expression was observed at low levels in hepatocytes, the sinusoidal epithelium and the sinusoidal lumen. Bcl-2 expression was positive in hepatocytes but negative in the sinusoidal endothelium and lumen. TNF-α expression was positive in hepatocytes and sinusoidal epithelial cells, as well as in the sinusoidal lumen, although it was weak in some regions.

In the BPA group, Bax expression was positive in the sinusoidal endothelium and hepatocytes, but negative in the sinusoidal lumen. Bcl-2 expression was observed at low levels in hepatocytes and the sinusoidal endothelium and was negative in the sinusoidal lumen. TNF-α expression showed intense immune reactivity in hepatocytes and the sinusoidal lumen, particularly in the endothelium. In the UD100 group, Bax expression was low in hepatocytes and negative in the sinusoidal endothelium and lumen. Bcl-2 expression was observed in both the sinusoidal endothelium and the hepatocytes.

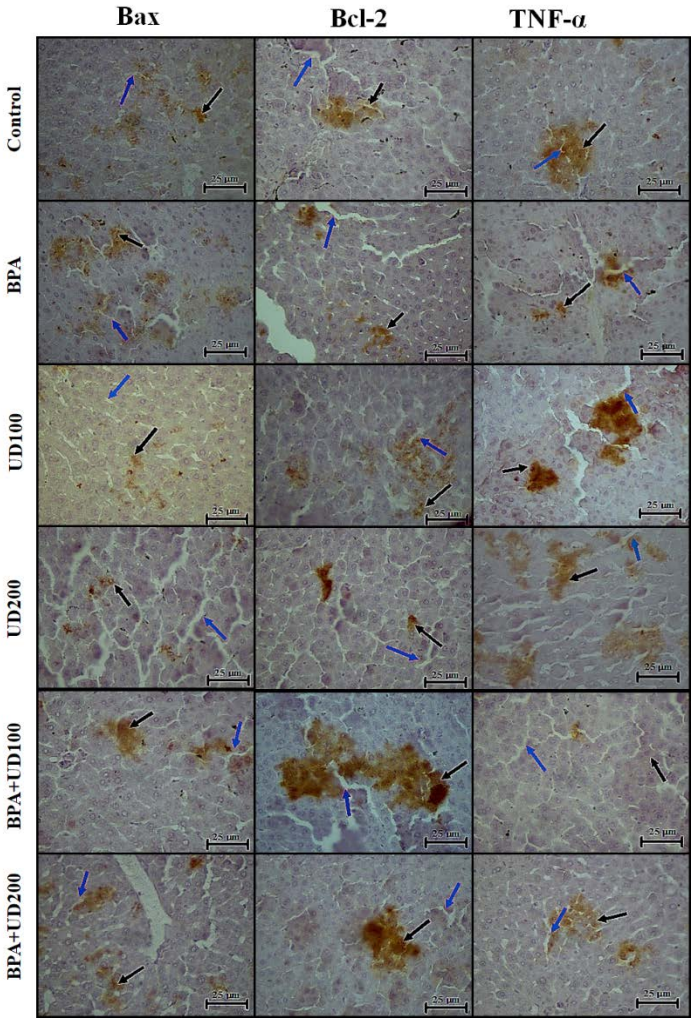


Figure 3. Immunohistochemical staining of liver sections. Bax, Bcl-2, and TNF-α protein expression.

Black arrow: Hepatocyte, blue arrow: Sinusoid. (Scale bar: 25 µm, Bax, Bcl-2, and TNF-α immunostaining, magnification: 40x). (BPA: Bisphenol A; UD: Urtica dioica; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; TNF-α: Tumor necrosis factor-α).

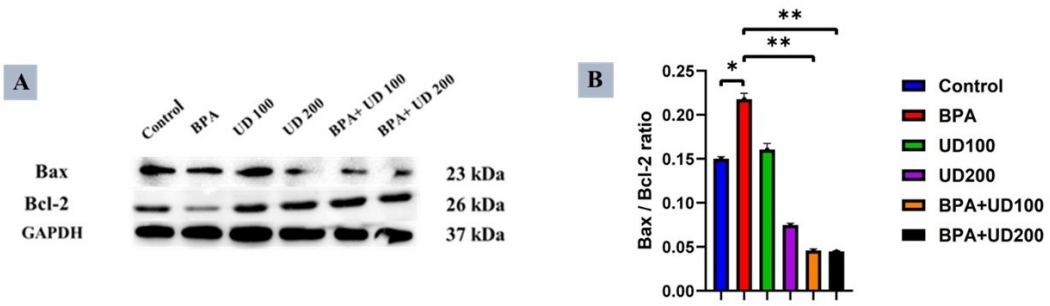


Figure 4. Bax/Bcl-2 protein expression ratio in liver tissues of the control, BPA, UD100, UD200, BPA+UD100, and BPA+UD200 groups. A. Quantification of band optical densities for Bax, Bcl-2, and GAPDH proteins. B. Bars represent the mean±SD of the experiments.

(*p<0.05, compared to the control; **p<0.05, compared to the BPA). (BPA: Bisphenol A; UD: Urtica dioica; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase).

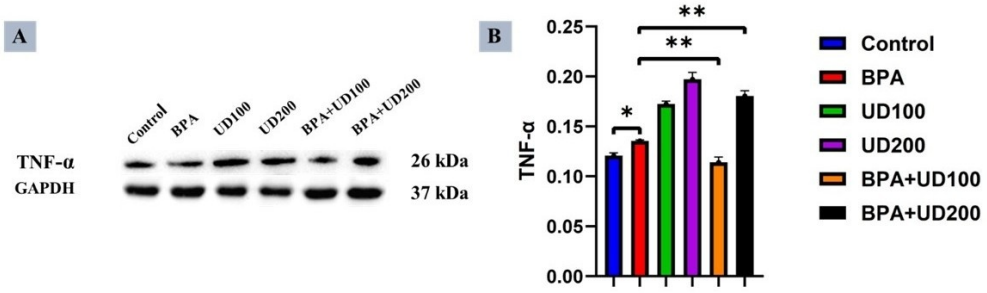


Figure 5. TNF-α expression levels in liver tissues of the control, BPA, UD100, UD200, BPA+UD100, and BPA+UD200 groups. A. Quantification of band optical density for TNF-α and GAPDH proteins. B. Bars represent the mean±SD of the experiments.

(*p<0.05, compared to the control; **p<0.05, compared to the BPA). (BPA: Bisphenol A; UD: Urtica dioica; TNF-α: Tumor necrosis factor-α; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase).

In the UD200 group, Bax expression was negative in the hepatocytes, the sinusoidal endothelium and the sinusoidal lumen. Bcl-2 expression was positive in hepatocytes but negative in the sinusoidal endothelium. In the UD100 and UD200 groups, TNF-α expression was positive in liver epithelial cells, the sinusoidal endothelium and the sinusoidal lumen. In the BPA+UD100 group, Bax expression was positive in liver epithelial cells, the sinusoidal endothelium and the sinusoidal lumen. Bcl-2 expression was positive in the sinusoidal endothelium and hepatocytes. TNF-α expression was negative in liver epithelial cells and the sinusoidal endothelium. In the BPA+UD200 group, Bax expression was positive in the sinusoidal endothelium and hepatocytes. Bcl-2 expression was positive in liver epithelial cells but negative in the sinusoidal endothelium and lumen. TNF-α expression was positive in liver epithelial cells, the sinusoidal endothelium and the lumen.

Western blot results

Figure 4 and Figure 5 present the expression levels of Bax, Bcl-2 and TNF-α proteins in liver tissues. The Bax/Bcl-2 protein expression ratio was significantly

higher in the BPA-treated group than in the control group ($p<0.05$). However, this ratio was significantly lower in the 100 mg/kg and 200 mg/kg UD treatment groups than in the BPA group ($p<0.05$). Additionally, this ratio was found to be lower solely in groups treated with UD at 100 and 200 mg/kg compared to the BPA group (Figure 4B). TNF-α expression levels were significantly higher in the BPA group than in the control group, and significantly lower in the BPA+UD100 group ($p<0.05$). TNF-α protein expression levels were observed to be higher than those in the control group in the UD100, UD200, and BPA+UD200 groups (Figure 5B).

DISCUSSION

Apoptosis is a highly critical process for the survival, development, and functioning of multicellular organisms. Dysregulation of apoptosis is associated with numerous diseases, ranging from cancer to degenerative disorders[21]. The Bcl-2 protein is an anti-apoptotic protein. It localizes to the outer mitochondrial membrane, preventing cytochrome c release and inhibiting programmed cell death[22]. The pro-apoptotic protein Bax, a member of the Bcl-2 family, promotes apoptosis by competing with Bcl-2. Bax is upregulated by p53 and has been reported to be involved in p53-mediated apoptosis[23]. It has been reported that BPA exposure may increase the hepatocellular apoptosis rate in the liver[24]. Similar to our study results, Keleş et al. demonstrated an increase in Bcl-2 levels in damaged liver tissue following UD treatment compared to the damage group[12]. TNF-α is a pro-inflammatory cytokine that plays a critical role in autoimmune diseases. In the liver, it mediates hepatocellular death. It has also been reported to be associated with autoimmune hepatitis (AIH), viral hepatitis, sterile inflammation, and hepatocellular carcinoma (HCC)[10]. Similar to the results of the present study, it has been demonstrated by conducted studies that TNF-α level increased as a result of BPA administration[5,9]. In this study, the aim was to suppress apoptosis and inflammation induced by BPA exposure in liver tissue and to improve tissue damage through UD treatment. Exposure to BPA, an endocrine-disrupting chemical, is inevitable due to its widespread use[24]. Studies have reported that this lipophilic substance causes damage to the liver, which is responsible for detoxifying xenobiotics, as well as various tissues, by reducing antioxidant enzyme levels, causing lipid peroxidation, and inducing apoptosis[24-26]. Fawzy and Negm reported that BPA exposure in liver tissue leads to an increase in Kupffer cells, dilation of blood sinusoids, and the presence of pyknotic nuclei and hyaline formations among hepatocytes[4]. BPA has also been reported to enhance inflammatory cell infiltration, nuclear aggregation, and cell necrosis[1]. Similarly, Uzunhisarcikli and Aslanturk reported that the liver tissues of rats exposed to BPA exhibited inflammatory cell infiltration, congestion, necrosis, and loss of cellular architecture[25]. BPA has also been reported to cause microvesicular steatosis and erythrocyte accumulation[6]. In another study, BPA was found to induce sinusoidal congestion, dilation of hepatic sinusoids between hepatic cords, irregularities in hepatic cords, and degeneration of hepatocytes[2,8]. BPA exposure was further shown to reduce total liver volume, hepatocyte count, and the nuclear and cytoplasmic volume of hepatocytes[27]. In our present study, BPA exposure was observed to cause disruption in the radial structure of hepatocyte cords, morphological alterations in hepatocytes, nuclear loss, and irregularities in the lumen of the central vein.

UD administration has been shown to improve the activity levels of key hepatic enzymes involved in glucose metabolism and to prevent weight loss[16]. As a result of the UD treatment, it was observed that white blood cells spread around the liver, hepatocytes arranged evenly and in normal size, and blood particles containing numerous widely spread cover cells were present[28]. UD has been reported to exert a protective effect against damage such as periportal and parenchymal inflammatory cell infiltration[29]. Furthermore, UD has been shown to significantly improve the histological structure of the liver, as well as biochemical and lipid profiles, while reducing the inflammatory process. It has been found that it provides protection against hepatotoxicity by neutralizing reactive oxygen species (ROS)[30]. In a similar study, UD was found to alleviate hepatocyte necrosis and restore the normal morphological features of liver tissue[31]. In the current study, it was observed that UD treatment against BPA-induced damage improved the radial arrangement of hepatocyte cords and enhanced the definition of hepatocyte boundaries and nuclei. It was also determined that it supported histopathological healing by providing regularity in the central vein lumen and definition in epithelial cell nuclei.

One of the most important limitations of this study is that the triggering of ROS production by the

increased inflammation following BPA-induced liver damage, and the resulting decrease in antioxidant enzyme levels, have not been demonstrated in vivo. It has been previously reported that elevated levels of pro-inflammatory cytokines such as TNF-α play a role in inducing apoptosis and causing tissue damage by increasing ROS production. Furthermore, although it has been demonstrated that UD application is effective in improving tissue damage by suppressing apoptosis and inflammation in BPA-induced liver toxicity, another limitation of the study is that the different pathways involved in this improvement have not been shown at the molecular level. Additionally, the not determining of the levels of aminotransferase group enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), which are known as indicators of liver damage, is another limitation. As a result, investigating the different molecular mechanisms that UD may play a role in the improvement of liver damage indicates the need for further studies.

In conclusion, this study elucidates that UD is beneficial in ameliorating histopathological and ultrastructural alterations induced by BPA in liver tissue by suppressing apoptosis and inflammation, owing to its antioxidant effects. However, further studies are required before definitive conclusions can be drawn regarding its potential effects on humans. UD may also be considered a beneficial dietary agent in mitigating hepatic toxicity caused not only by BPA but also by other xenoestrogenic compounds.

Author Contributions: Concept/Design : GPŞ, SAŞ; Data acquisition: GPŞ, SAŞ; Data analysis and interpretation: GPŞ, GK, SAŞ; Drafting manuscript: GPŞ, GK, SAŞ; Critical revision of manuscript: GPŞ, GK, SAŞ; Final approval and accountability: GPŞ, GK, SAŞ; Technical or material support: GPŞ, GK, SAŞ; Supervision: GPŞ; Securing funding (if available): n/a.
Ethical Approval: The experimental protocol used in this study was approved by the Local Ethics Committee of Animal Experiments in Hatay Mustafa Kemal University at its meeting on 24/06/2025, under decision number 2025/05-07.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support
Informed Consent: Informed consent was obtained from all participants.

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