https://doi.org/10.52973/rcfcv-e35602 Revista Científica, FCV-LUZ / Vol. XXXV

Ursodeoxycholic acid alleviates *Xanthium Strumarium* induced hepatic and renal toxicity in rats by inhibiting mitochondrial pore opening

El ácido ursodesoxicólico alivia la toxicidad hepática y renal inducida por *Xanthium strumarium* en ratas al inhibir la apertura de los poros mitocondriales

Zeliha Keskin Alkaç[1]*, Fatih Ahmet Korkak[2], Gürdal Dağoğlu[2], Yesari Eröksüz[3], Sadettin Tanyıldızı[2]

[1]Firat University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology. Elaziğ, Türkiye.
[2]Firat University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology. Elaziğ, Türkiye.
[3]Firat University, Faculty of Veterinary Medicine, Department of Pathology. Elaziğ, Türkiye.
**Corresponding author: zkeskin@firat.edu.tr*

ABSTRACT

In *Xanthium strumarium* toxicity, mitochondrial dysfunction resulting from the opening of mitochondrial pores is identified as the primary mechanism responsible for liver and kidney damage. Ursodeoxycholic acid is known to block mitochondrial pore opening; therefore, this study aims to elucidate the time–dependent therapeutic effect of ursodeoxycholic acid on mitochondrial damage and associated liver and kidney injury in response to *X. strumarium* exposure. Following the extraction process, Sprague–Dawley rats were administered *X. strumarium* seed extract (100 $g \cdot kg^{-1}$) via gavage. Ursodeoxycholic acid was administered via oral gavage 6 hours following the administration of the extract, with continued administration over a 7-day period. In conclusion, the toxic effect of *X. strumarium* was mitigated by ursodeoxycholic acid, which reduced ATP synthase expression, oxidative damage, mitochondrial Ca^{2+} concentration, and the opening of mitochondrial pores. Ursodeoxycholic acid mitigated the histopathological toxicity induced by *X. strumarium*, resulting in a reduction in blood glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, blood urea nitrogen, and creatine phosphokinase levels that were closer to control levels. Findings obtained indicate that ursodeoxycholic acid, a blocker of mitochondrial pore opening, can prevent mitochondrial dysfunction and minimize *X. strumarium* toxicity.

Key words: *Xanthium strumarium* L.; ursodeoxycholic acid; mPTP; mitochondrial Ca^{2+}; immunohistochemistry

RESUMEN

En la toxicidad de *Xanthium strumarium*, la disfunción mitocondrial resultante de la apertura de los poros mitocondriales, se identifica como el mecanismo principal responsable del daño hepático y renal. Se sabe que el ácido ursodesoxicólico bloquea la apertura de los poros mitocondriales; por lo tanto, este estudio tiene como objetivo dilucidar el efecto terapéutico dependiente del tiempo del ácido ursodesoxicólico sobre el daño mitocondrial y las lesiones hepáticas y renales asociadas en respuesta a la exposición a *X. strumarium*. Después del proceso de extracción, a las ratas Sprague–Dawley se les administró extracto de semilla de *X. strumarium* (100 $g \cdot kg^{-1}$) por sonda. El ácido ursodesoxicólico se administró por sonda oral 6 horas después de la administración del extracto, y se continuó la administración durante un período de 7 días. En conclusión, el efecto tóxico de *X. strumarium* fue mitigado por el ácido ursodesoxicólico, que redujo la expresión de la ATP sintasa, el daño oxidativo, la concentración mitocondrial de Ca^{2+} y la apertura de los poros mitocondriales. El ácido ursodesoxicólico mitigó la toxicidad histopatológica inducida por *X. strumarium*, lo que resultó en una reducción de los niveles de glucosa en sangre, alanina aminotransferasa, aspartato aminotransferasa, fosfatasa alcalina, lactato deshidrogenasa, nitrógeno ureico en sangre y creatina fosfoquinasa que estaban más cerca de los niveles de control. Los hallazgos obtenidos indican que el ácido ursodesoxicólico, un bloqueador de la apertura de los poros mitocondriales, puede prevenir la disfunción mitocondrial y minimizar la toxicidad de *X. strumarium*.

Palabras clave: *Xanthium strumarium* L.; ácido ursodesoxicólico; mPTP; Ca^{2+} mitocondrial; inmunohistoquímica

Received: 25/12/2024 Accepted: 26/02/2025 Published: 22/04/2025

INTRODUCTION

Xanthium strumarium L., a member of the Asteraceae family, is a wild and annual herbaceous plant that typically flourishes in humid regions [1]. *Xanthium strumarium* is a toxic plant, and its seeds and germinated cotyledons contain toxic compounds [2]. As a result of phytochemical analysis, it was found that carboxyatractyloside (CATR) and atractyloside (ATR) are the compounds responsible for the toxicity of the plant [2].

Xanthium species poisoning is a relatively uncommon occurrence worldwide, with the majority of documented cases involving domestic animals such as pigs, cattle, and sheep [3, 4]. In particular, the ingestion of poisonous plants mixed with cereals or the infestation of silage by poisonous plants represents the primary cause of poisoning in animals [5]. It is noteworthy that Xanthium poisoning is not restricted to domestic animals; fatal cases have also been observed in humans [6, 7]. The poisoning of humans by the Xanthium species typically occurs as a consequence of the pharmacological utilization of the plant or inadvertent ingestion by children [6, 7]. The majority of poisoning cases in children result in fatality [7, 8].

It has been determined that CATR and ATR exert their toxic effects by binding to the adenine nucleotide transporter protein (ANT), which is located in the mitochondrial inner membrane [9]. The binding of CATR and ATR to the ANT protein at the – c position has been demonstrated to facilitate the opening of the mitochondrial permeability transition pores (mPTP) [9, 10]. mPTP opening is associated with mitochondrial Ca^{2+} levels. Increased mitochondrial Ca^{2+} binds to its receptors in the inner mitochondrial membrane, where it promotes mPTP opening [9]. The opening of the mPTP has been reported to result in the necrosis or apoptosis of specific cell types [9]. Moreover, the binding of these toxic compounds to ANT impedes the transport of adenosine diphosphate to the mitochondrial membrane. Consequently, oxidative phosphorylation is disrupted since adenosine triphosphate cannot be produced [11]. Blocking of oxidative phosphorylation results in an intracellular energy crisis, which ultimately leads to cell death [12].

Ursodeoxycholic acid (UDCA) is a hydrophilic bile salt that is commonly utilized in clinical practice for the dissolution of cholesterol gallstones and the improvement of cholestatic liver disease [13]. Recent studies have proven that UDCA exerts antiapoptotic effects in pathological conditions associated with mitochondrial dysfunction by inhibiting the opening of the mPTP [14, 15]. However, to date, no evidence exists to suggest that UDCA is an effective treatment for poisoning caused by the *Xanthium* species. The principle of this study is to demonstrate that UDCA offers protection against the toxic effects of *X. strumarium* by inhibiting the pathological opening of the mPTP in mitochondria isolated from rats that have been challenged with *X. strumarium*.

MATERIAL AND METHODS

Extraction of *Xanthium strumarium*

The seeds of *Xanthium strumarium* were removed and pulverized using an IKAA11 basic grinder (Sigma Aldrich, St. Louis, MO, USA). The seed powder was subjected to a boiling process with water at a temperature of 100°C for a period of 30 min. Subsequently, the mixture was passed through filter papers (S&HLabware–125 mm) and concentrated with the assistance of an evaporator (NEUBERGER, Germany) maintained at 40-45°C.

In order to calculate the dose of the extract to be administered to the rats (*Rattus norvegicus*) in the experiment, the ATR levels in the extract were determined. To this end, 2 mL of hydrochloric acid (HCl) (2 $mol \cdot L^{-1}$) was added to 1 mL of extract, vortexed (VELP Scientifica APX–153, VELP China Co. Ltd. Shanghai, China), and kept in the oven (Nüve FN–500, Nüve, Ankara, Türkiye) at 25°C for 12 h. Subsequently, the sample was subjected to centrifugation (Hettich UNIVERSAL 320 R, Ankara, Türkiye) at 3500×g for a period of 5 min, after which the supernatant was taken to a separate clean tube.

Later, 2 mL of ethyl acetate was added to the hydrolyzate, which was then subjected to centrifugation at 3500×g for a period of 5 min. The resulting ethyl acetate phase was transferred to another tube. The ethyl acetate process was repeated five times. The collected organic extract was then dried at 45°C under nitrogen gas. The residue was then subjected to a derivatization process involving the addition of 100 µl of Pyridine and 100 µl of (trimethylsilyl)imidazole (TMSI) at 100°C for a period of 2 h. Following a 2 h derivatization period, a 2 µl of the sample volume was injected into a gas chromatography mass spectrometry (GC–MS) (Shimadzu, GCMS–QP2010, Tokyo, Japan) apparatus [16]. The results of the analysis indicated that the ATR level in *Xanthium strumarium* seeds (XSS) was 4 $mg \cdot g^{-1}$.

Animal treatments

The rats were sourced from the Experimental Research Center at Fırat University. All experiments were conducted in accordance with the guidelines set forth by the Local Ethics Committee for Animal Experiments of Fırat University (2019/14). Experiments were performed on female Sprague Dawley rats (7 weeks old, weight of 200 ± 10 g). The rats were housed under standard conditions, including a consistent temperature and ventilation, a 12–hour light/12–hour dark cycle, and access to a standard rat diet and water *ad libitum*.

The XSS extract was administered to the rats in the XSS group via gavage at a dose of 100 $mg \cdot kg^{-1}$ at the commencement of the study. The control group was administered saline via gavage on a daily basis. Six h after the induction of toxicity, the rats in the UDCA and XSS+UDCA groups were administered UDCA at a dose of 20 $mg \cdot kg^{-1}$ via gavage, with the administration repeated at the same time each day. At 6, 24, 72, 120, and 168 h following the initial administration of UDCA, 5 rats from each group were euthanized.

Serum biochemistry measurement

Blood samples were obtained from decapitated rats and centrifuged at 3500×g for 15 min, after which the serum was removed. Analyses of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine phosphokinase (CK), lactate dehydrogenase (LDH), creatinine, and blood urea nitrogen, were conducted in the Biochemistry Laboratory of Fırat University Hospital using an automated analyzer (Olympus AU-660, Tokyo, Japan).

Tissue biochemistry measurements

Spectrophotometric analyses were conducted to determine the levels of superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), and protein. Following homogenization of the kidney and liver tissues, the resulting homogenate was analyzed for MDA using the method stated by Ohkawa *et al.* [17]. The supernatant obtained from the homogenate after centrifugation was analyzed for GSH activity using the principle of reduction of 5,5–dithio–bis[2–nitrobenzoic acid] (DTNB) by sulfhydryl compounds [18]. The measurement of SOD activity was based on the reduction of nitrobluetetrazolium (NBT) by superoxide (O_2^-) produced by the xanthine/xanthine oxidase system [19]. The protein content of the supernatant was determined using the method stated by Lowry *et al.* [20].

Measurement of mitochondrial parameters

Mitochondria isolation

The liver and kidney were homogenized with buffer A (1:10, g/v), which consisted of 70 mM sucarose, 220 mM mannitol, 2.0 mM ethylene diamine tetra acetic acid (EDTA), 5.0 mM 3–morpholine propane sulfonic acid, and 0.5% bovine serum albumin (BSA), and the pH was adjusted to 7.4. The homogenization (DAIHAN Scientific HG–15D, Türkiye) was conducted at 4°C. Subsequently, the homogenate was subjected to a 10 min centrifugation at 1,000×g at 4°C, after which the supernatant was subjected to a second centrifugation under identical conditions. Subsequently, buffer B (obtained by a 7.5–fold dilution of buffer A without BSA, with a pH of 7.4) was added to the supernatant, and the mixture was subjected to centrifugation at 8000×g at +4°C for 20 min [21]. The residual mitochondrial pellet at the base of the tube was resuspended in buffer B and subjected to mitochondrial protein determination [20].

Measurement of mitochondrial Ca^{2+}

A total of 200 µl of the mitochondrial preparation, comprising 2.0 mg protein, was combined with 2 mL nitric acid and 1 mL ultrapure water and it was left at room temperature for 1 h. Subsequently, 1 mL of a 20 $g \cdot L^{-1}$ lanthanum oxide solution was added to the mixture. Once the final volume of the mixture had been made up to 10 mL with 1% nitric acid, the absorbance values were then measured by means of a flame atomic spectrophotometer (AAS, PerkinElmer, Analyst 800, Norwalk, CT, USA) [22].

Measurement of mPTP

Upon opening of the mPTP, the inner membrane of mitochondria becomes permeable for mannitol and sucrose. This is determined by a decrease in absorbance in the spectrophotometer reading at 540 nm. Mitochondria obtained as pellets was suspended in 3 mL of cold assay medium (230 mM mannitol, 70 mM sucrose, 3 mM HEPES, pH 7.4), and the protein concentration was measured [20]. Subsequently, the protein amount was adjusted to 0.3 $mg \cdot mL^{-1}$ using the cold test medium; 3 mL of cold test medium was added to 1 mL of this suspension, and the absorbance was measured at 540 nm using UV–spectrophotometry (VWR-3100PC UV Spectrophotometer) [23].

Measurement of ATR levels in tissues

A volume of 0.5 mL of the serum sample was taken to a clean tube, followed by the addition of 1 mL of acetone. Liver and kidney tissues were homogenized with phosphate buffered saline (PBS) [8.06 g NaCl, 0.22 g KCl, 1.15 g Na_2HPO_4, 0.20 g KH_2HPO_4, 1 L distilled water, pH:4.5] at a ratio of 1:4 ($g \cdot v^{-1}$). All prepared samples were subjected to centrifugation at 3500×g for a period of 5 min. The upper solutions were then dried in nitrogen gas at 45°C. The residues were dissolved in 1 mL of distilled water, and 2 mL of HCl (2 $mol \cdot L^{-1}$) was added and left for 12 h at room temperature. Once the designated waiting period had elapsed, the solution was subjected to centrifugation, after which the supernatant was extracted with 2 mL of ethyl acetate. The extraction with ethyl acetate was repeated five times, and the resulting organic extracts were subsequently dried under nitrogen gas. The final residue was derivatized with 100 µl pyridine and 100 µl TMSI at 100°C for 2 h, and 2 µl was injected into GC–MS instrument [16, 24].

The GC–MS (Shimadzu, GCMS–QP2010, Tokyo, Japan) was employed for the purpose of determining ATR levels in tissues. The DB-1 capillary column (30 m × 250 µm in diameter, with a film thickness of 0.1 µm) and an SGE 10 µl injector were employed during the analysis. The detector temperature was set at 200°C, and the injector temperature was set at 250°C. The temperature of the column was initiated at 215°C and increased at a rate of 2.30°C·min^{-1} until it reached 310°C. The helium gas flow rate was set to 1.9 $mL \cdot min^{-1}$, and the samples were analyzed in splitless scanning mode, as described by Laurens *et al.* [16].

Histopathologic measurements

The tissues were fixed in 10% formalin solution, then underwent a series of dehydration steps involving alcohol and xylol, followed by paraffin embedding. From the paraffin blocks, sections 3–5 µm in thickness were obtained on positively charged slides using a rotary microtome (Leica RM2125, Wetzlar, Germany). The sections were subsequently subjected to staining with hematoxylin and eosin in a tissue staining machine (Leica Autostainer XL, Wetzlar, Germany) [25].

Immunohistochemical measurements

The Avidin–Biotin–Peroxidase Complex (ABC) procedure was employed for immunohistochemical measurements [26]. The primary antibody utilized was anti–ATP–synthase c (Abcam, 1/200). The standard staining procedure recommended by the manufacturer was followed using the chemicals provided in the ready immunohistochemistry kit. All sections were examined under a light microscope (Olymus BX43, Tokyo, Japan) at 20× magnification. The immunohistochemical staining score was evaluated according to the following criteria; 1: light, 2: moderate, 3: severe.

Statistical Evaluation

The statistical evaluations were conducted utilizing the "IBM SPSS Statistics 21" software package. A one–way analysis of variance (ANOVA) was employed to ascertain the existence of differences between groups at varying points in time, with a *post hoc* Tukey test utilized for intra–group comparisons. All data are reported as mean ± standard error of the mean (mean ± SEM). Data with a *P* value less than 0.05 were evaluated as statistically significant.

RESULTS AND DISCUSSION

TABLE I presents the distribution of ATR levels in blood, kidney, and liver tissues over time in rats administered XSS extract. The highest concentration of ATR was observed at the 24–h mark in all examined tissues from both the XSS and XSS+UDCA groups. At the 168th h, no ATR was detected in the body. The administration of UDCA in conjunction with XSS extract did not result in a statistically significant alteration in ATR levels within the examined tissues (P>0.05).

TABLE I
Time profile of blood, liver and kidney ATR levels in rats administered XSS extract

Groups	6th	24th	72th	120th	168th
Blood ATR (μg·mL^{-1})					
Control	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}
UDCA	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}
XSS	0.415 ± 0.019^{b}	2.036 ± 0.019^{b}	1.560 ± 0.105^{b}	0.099 ± 0.011^{a}	0.000 ± 0.000^{a}
XSS+UDCA	0.576 ± 0.035^{b}	2.149 ± 0.078^{b}	1.407 ± 0.108^{b}	0.076 ± 0.010^{a}	0.000 ± 0.000^{a}
Liver ATR (μg·g^{-1})					
Control	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}
UDCA	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}
XSS	0.478 ± 0.022^{b}	2.321 ± 0.133^{b}	1.141 ± 0.043^{b}	0.131 ± 0.014^{a}	0.000 ± 0.000^{a}
XSS+UDCA	0.321 ± 0.090^{b}	2.158 ± 0.08^{b}	1.600 ± 0.097^{b}	0.092 ± 0.010^{a}	0.000 ± 0.000^{a}
Kidney ATR (μg·g^{-1})					
Control	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}
UDCA	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}
XSS	0.330 ± 0.020^{b}	1.704 ± 0.234^{b}	1.380 ± 0.036^{b}	0,145 ± 0.011^{a}	0.000 ± 0.000^{a}
XSS+UDCA	0.292 ± 0.009^{b}	2.126 ± 0.047^{c}	1.385 ± 0.122^{b}	0.241 ± 0.143^{a}	0.000 ± 0.000^{a}

One–way ANOVA and Tukey were performed for group comparisons. Values reported are mean ± SEM, ^{a,b,c}: Distinction between groups shown with different letters in the same column are statistically significant (P<0.05). ATR: Atractyloside, XSS: *Xanthium strumarium* seeds, UDCA: Ursodeoxycholic acid

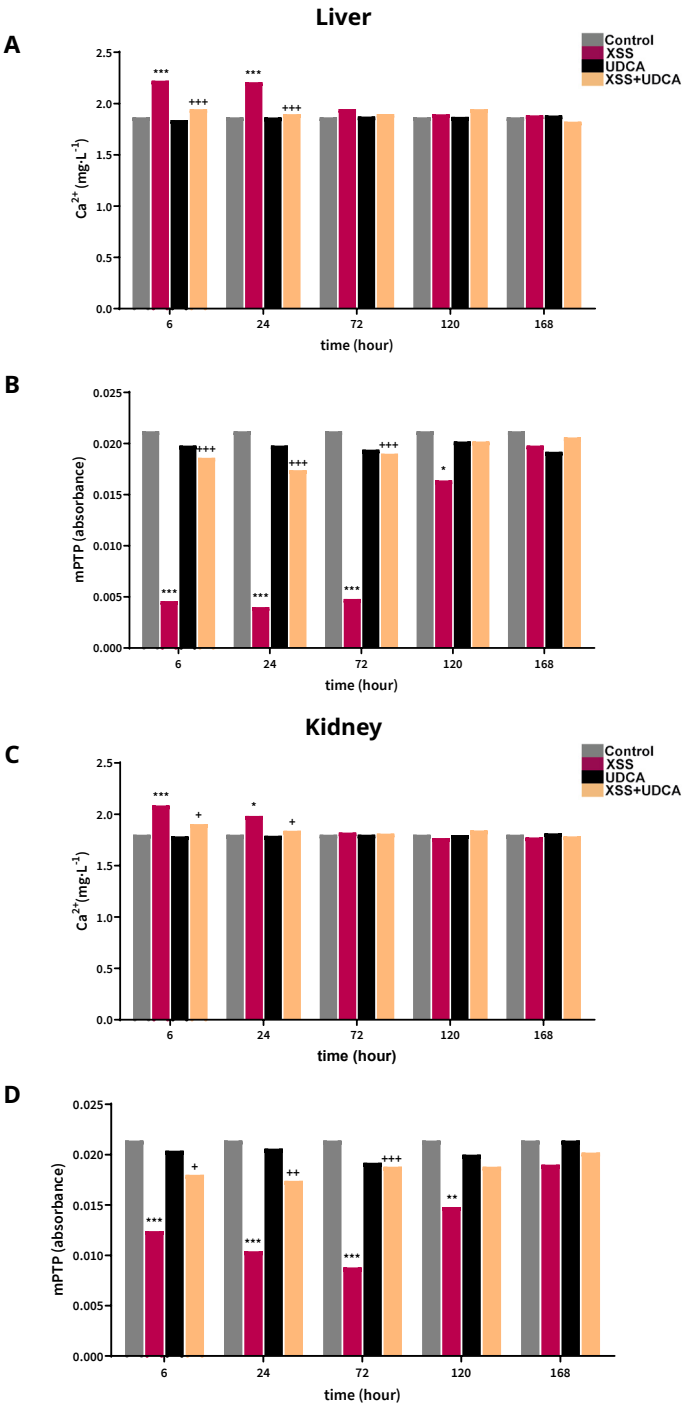

FIGURE 1. Effect of ursodeoxycholic acid on mitochondrial Ca^{2+} levels and mitochondrial permeability transition pore opening in liver and kidney tissues in rats with *Xanthium strumarium* toxicity. XSS: *Xanthium strumarium* seed extract, UDCA: Ursodeoxycholic acid, mPTP: mitochondrial permeability transition pore. [A: liver Ca^{2+}, B: liver mPTP, C: kidney Ca^{2+}, D: kidney mPTP]. *P<0.05, **P<0.01, ***P<0.001 compared with the control group; +P<0.05, ++P<0.01, +++P<0.001 compared with the XSS group

Increased oxidative damage in cells due to exposure to toxic compounds and different pathological conditions increases Ca^{2+} levels in mitochondria and decreases mitochondrial membrane potential. These events result in the opening of mPTPs, which are located in the mitochondrial inner membrane and permit the passage of only a limited number of substances under physiological conditions. The opening of mPTPs allows for the passage of water and solutes into the mitochondria, thereby disrupting the pressure equilibrium between the cytoplasm and mitochondria. An increase in the volume of the mitochondrial matrix results in the rupture of the outer mitochondrial membrane [27, 28]. After XSS extract treatment in rats, we analyzed the absorption of Ca^{2+} and mPTP in mitochondria isolated from liver and kidney tissues. The results demonstrated a notable elevation in mitochondrial Ca^{2+} levels in both tissues at both the 6– and 24– hour time points (P<0.05). Absorbance values, which are indicators of mitochondrial pore opening, decreased at all times examined, although they were significant at 6, 24, 72, and 120 h. (P<0.05). In rats with XSS toxicity, treatment with UDCA resulted in a notable reduction in mitochondrial Ca^{2+} levels and a marked inhibition of mPTP opening, as illustrated in FIG. 1.

In experimental previous studies conducted on rats, it was reported that exposure to *Xanthium* species plants at varying doses resulted in elevated levels of MDA, a marker of oxidative damage in kidney and liver tissues, and a reduction in antioxidant enzymes [29]. The present study yielded the statistically significant result that MDA levels in kidney and liver tissues of XSS–treated

rats increased at all hours ($P<0.05$), a finding that aligns with the results of previous researchers in this [25, 29, 30]. Furthermore, the levels of antioxidant enzymes, SOD and GSH, were observed to decline, with a more pronounced reduction observed in the liver ($P<0.05$, FIG.2). This phenomenon may be attributed to the fact that ATR and CATR induce lipid peroxidation and elevate MDA levels in kidney and liver tissues. The rise in oxidative stress was linked to an elevation in Ca^{2+} within the mitochondria [27, 28]. And, an elevation in mitochondrial Ca^{2+} amount was observed at 6 and 24 h in mitochondria extracted from the livers and kidneys of rats that had been administered XSS.

The present study demonstrates that an increase in mitochondrial Ca^{2+} concentration, which is associated with oxidative stress, results in the opening of mPTPs. This phenomenon was observed across all examined time points. The opening of mPTPs is a complex process that is also linked to ANT. ANT protein plays an important role in channel opening as a component of the mPTP [31]. Additionally, ATR and CATR, which are responsible for the toxicity of Xanthium strumarium, stimulate the opening of mPTP by binding to ANT with a high affinity [9]. UDCA, which has been the subject of investigation as a potential therapeutic agent in the context of *Xanthium* toxicity, was observed to reduce MDA levels in the rats that were administered it, bringing them to levels that were similar to those observed in the control group. Furthermore, the UDCA group demonstrated a notable elevation in antioxidant enzyme levels in comparison to the XSS group (FIG. 2).

Xanthium strumarium seeds (XSS) caused a significant increase in ATP–synthase activity as a result of immunohistochemical examinations. ATP–synthase is an important enzyme located in the inner membrane of mitochondria. It catalyzes the synthesis of ATP from ADP and inorganic phosphate (Pi) under physiological conditions [32]. However, in pathological conditions accompanied by mitochondrial dysfunction, the synthesis of ATP is impaired, and the available ATP is broken down by the catalytic activity of the ATP–synthase enzyme and transported to the mitochondrial matrix. This process facilitates the transport of protons to the

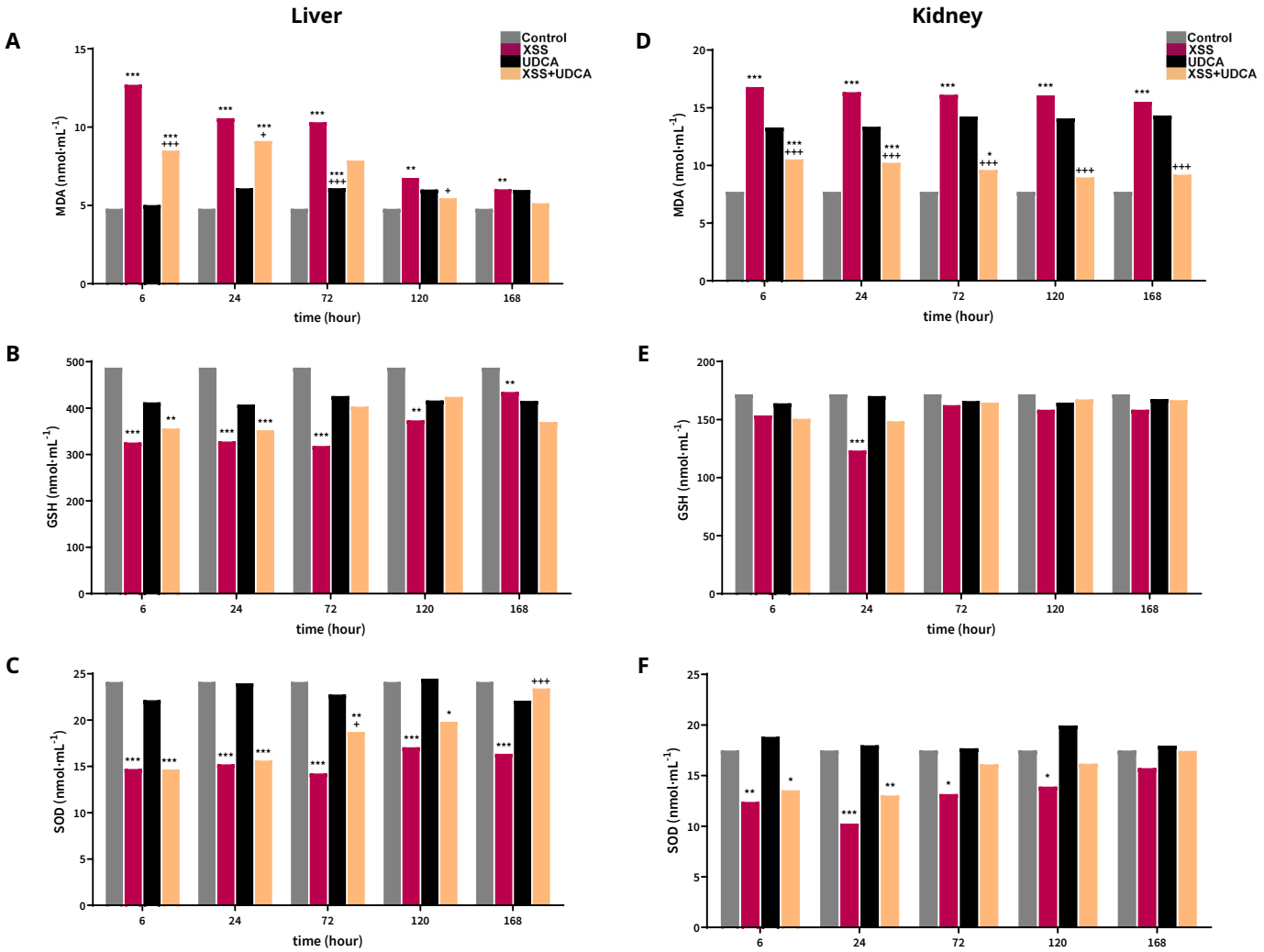

FIGURE 2. Effect of ursodeoxycholic acid on oxidative stress in liver and kidney tissues in rats with *Xanthium strumarium* toxicity. XSS: *Xanthium strumarium* seed extract, UDCA: Ursodeoxycholic acid. [A: liver malondialdehyde (MDA), B: liver reduced glutathione (GSH), C: liver superoxide dismutase (SOD), D: kidney MDA, E: kidney GSH, F: kidney SOD]. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with the control group; +$P<0.05$, +++$P<0.001$ compared with the XSS group

mitochondria [33, 34]. The elevated ATP–synthase activity observed in the XSS group (TABLE II and FIG. 3; $P<0.05$) is believed to be a consequence of the transport and breakdown of ATP into the mitochondria. This may be interpreted as an attempt to repair mitochondrial dysfunction. However, it was observed that UDCA treatment reduced the XSS–induced increase in ATP synthase activity in the liver tissues of rats ($P<0.05$).

TABLE II
Immunohistochemical intensity and distribution degrees of ATP–synthase in the livers of rats given *Xanthium strumarium* seed extract

	ATP–synthase (mean ± SEM)				
Groups	**6th**	**24th**	**72th**	**120th**	**168th**
Control	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}
XSS	2,400 ± 0,24^{b}	3,000 ± 0,00^{b}	1,000 ± 0,00^{b}	0,600 ± 0,24^{b}	0,600 ± 0,24^{b}
UDCA	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}
XSS+UDCA	1,200 ± 0,20^{c}	1,200 ± 0,20^{c}	0,600 ± 0,24^{bc}	0,000 ± 0,00^{a}	0,000 ± 0,00^{a}

One–way ANOVA and Tukey were performed for group comparisons. $^{a, b, c}$: Distinction between groups indicated with different letters in the same column are statistically significant ($P<0.05$).
XSS: *Xanthium strumarium* seeds, UDCA: Ursodeoxycholic acid

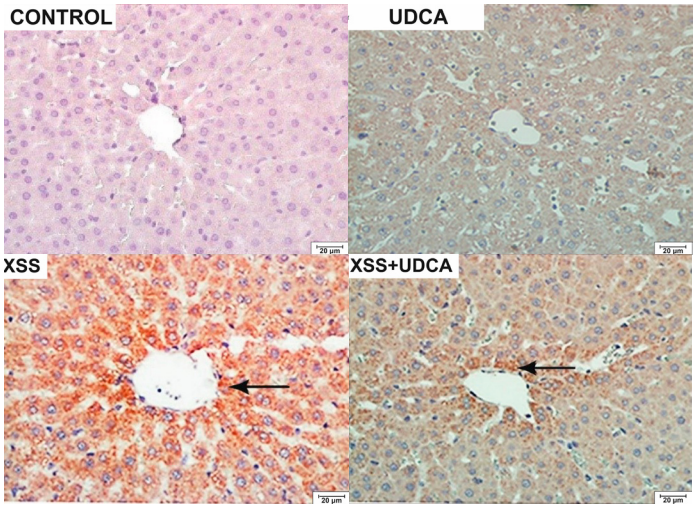

FIGURE 3. Severity and prevalence of immunohistochemical expression of ATP–synthase in the liver at 24 hours after ursodeoxycholic acid treatment in rats given *Xanthium strumarium* seed extract. XSS: *Xanthium strumarium* seeds, UDCA: Ursodeoxycholic acid. Horseradish Peroxidase technique; amino-9-ethyl carbazole chromogen and hematoxylin counterstaining, Bar: 20 µm

Oxidative stress represents a significant contributor to tissue damage, particularly within the liver. XSS–induced oxidative stress injury is mediated by the activation of lipid peroxidation, which impairs the antioxidant defense system and results in hepatorenal toxicity. UDCA has been demonstrated to reduce the formation of reactive oxygen species by modulating the activities of CAT and SOD. This mechanism contributes to the protection of cells against damage induced by oxidative stress. For these reasons, UDCA is a commonly utilized compound in the field of hepatology [35, 36]. UDCA plays a pivotal role in safeguarding cells from the detrimental effects of toxins, pharmaceuticals, and other hepatotoxic agents by enhancing the stability of plasma and mitochondrial membranes of hepatocytes [35, 37].

In recent years, potential therapeutic strategies targeting mitochondrial dysfunction have been investigated in the treatment of diseases and toxicities mediated by mitochondrial dysfunction. For this purpose, many compounds targeting mitochondria have been studied. One of these compounds, UDCA, is a good mitochondrial protective candidate with its ability to prevent pathological events such as accumulation of reactive oxygen species, collapse of mitochondrial membrane potential, and ATP depletion [38, 39]. UDCA is already an approved treatment for most cholestatic liver diseases [39].

In this study, UDCA, which possesses antioxidant properties, was observed to mitigate the rise in intracellular Ca^{2+} and oxidative stress, enhance SOD activity and GSH levels, and forestall mPTP opening in XSS toxicity in liver and kidney tissues. In this regard, UDCA demonstrated the capacity to safeguard cells against the deleterious effects of oxidative stress and mitochondrial damage. The observed reduction in ATP–synthase activity in XSS toxicity can be attributed to the antioxidant properties of UDCA, which have been demonstrated to prevent mitochondrial damage and maintain mitochondrial integrity.

The administration of XSS to rats resulted in a toxic effect, as evidenced by elevated levels of AST, ALP, LDH, ALT, CK, creatinine, and BUN, along with decreased blood glucose levels, when compared to the control group ($P>0.05$, FIG.4). The findings of this study are consistent with those of previous studies showing that XSS causes elevated liver and kidney function test results. [6, 40]. Elevated liver enzymes in serum indicate liver damage, while BUN and creatinine indicate kidney damage [35, 41]. Furthermore, XSS has been linked to disturbances in carbohydrate metabolism, as evidenced by its ability to reduce blood glucose levels and disrupt ATP production in cells by impeding oxidative phosphorylation [7]. UDCA's capacity to diminish the elevated AST, ALP, LDH, ALT, CK, creatinine, BUN, and decreased glucose levels resulting from XSS toxicity to levels approaching those observed in the control group is indicative of its hepatorenal protective effect.

Findings of the current study revealed that XSS resulted in histopathological alterations in the liver, encompassing degeneration, portal lymphohistiocytic infiltration, periportal infiltration, subcapsular necrosis and single cell necrosis (FIG.5). Similarly, other researchers have reported that XSS causes hepatotoxicity, with comparable histopathological findings [29, 41]. These findings demonstrated that XSS–induced hepatocyte injury occurs through multiple mechanisms, including impairment of mitochondrial function, alteration of energy metabolism, disruption of the lipid membrane of cells through oxidative damage, and induction of mPTP opening. The co–administration of UDCA and XSS was observed to prevent the histopathological effects induced by XSS. It appears that UDCA may offer protection against XSS–induced hepatotoxicity.

In order to ascertain the nature of the cell death induced by XSS, the TUNEL staining method was employed in conjunction with conventional histopathologic examinations. However, the number of apoptotic cells identified through TUNEL staining was limited in the XSS group (FIG.6).

Prior research has demonstrated that mPTP opening can result in not only apoptotic but also necrotic cell death [42, 43]. The

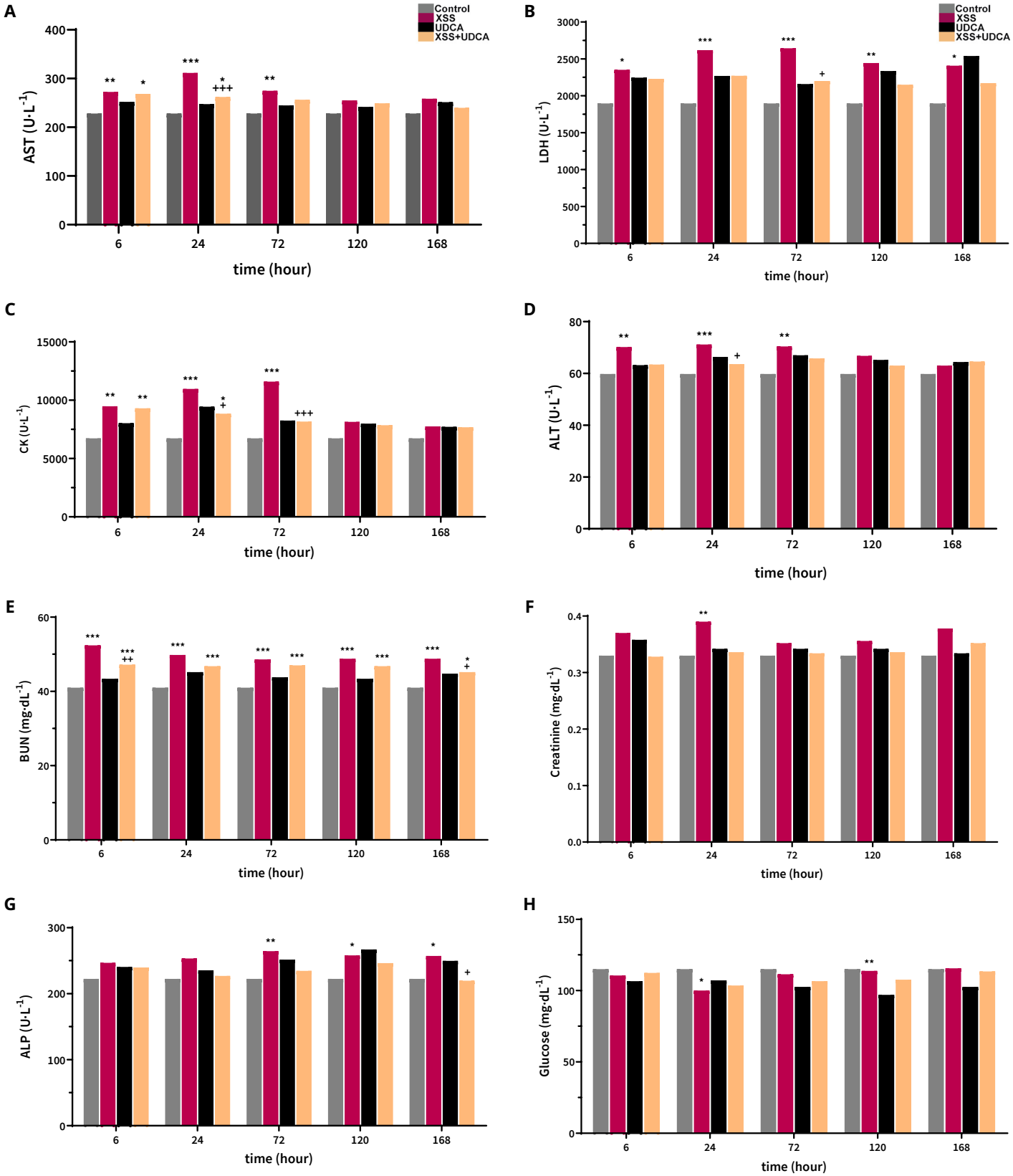

FIGURE 4. Effect of ursodeoxycholic acid on blood biochemistry in rats with *Xanthium strumarium* toxicity. XSS: *Xanthium strumarium* seed extract, UDCA: Ursodeoxycholic acid. [A: aspartate amino transferase (AST), B: lactate dehydrogenase (LDH), C: creatinine phosphokinase (CK), D: alanine amino transferase (ALT), E: blood urea nitrogen (BUN), F: Creatinine, G: alkaline phosphate (ALP), H: Glucose]. **P*<0.05, ***P*<0.01, ****P*<0.001 compared with the control group; +*P*<0.05 , ++*P*<0.01, +++*P*<0.001 compared with the XSS group

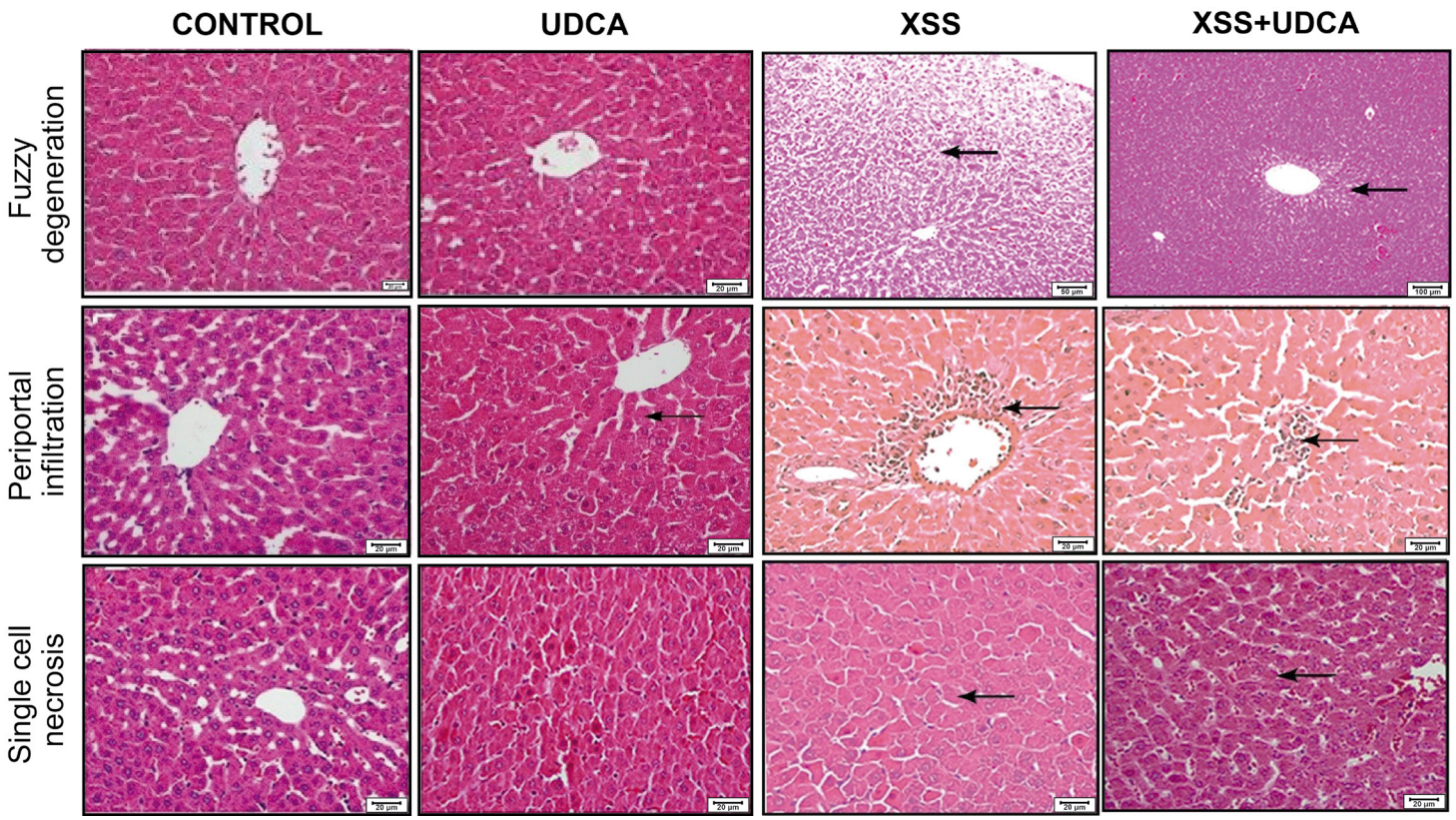

FIGURE 5. Effect of ursodeoxycholic acid on liver histopathology in rats with *Xanthium strumarium* toxicity. XSS: *Xanthium strumarium* seed extract, UDCA: Ursodeoxycholic acid. Histopathological scoring in experimental groups: (Fuzzy degeneration; control = (1), XSS = (3), XSS + UDCA = (1), Periportal infiltration; control = (0), XSS = (3), XSS+UDCA = (1), Single–cell necrosis; control = (0), XSS = (3), XSS + UDCA = (2))

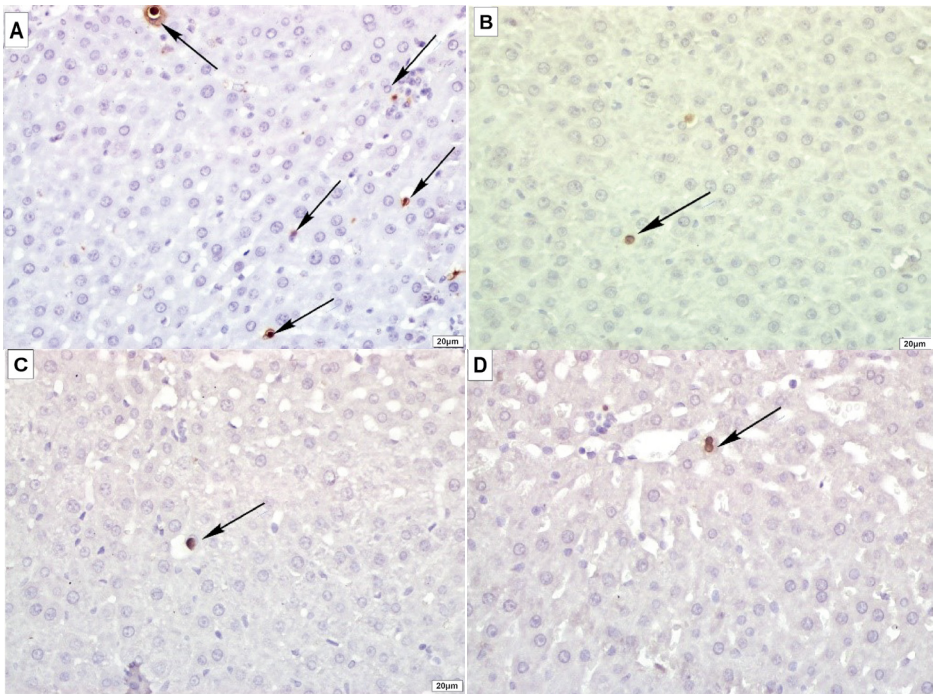

FIGURE 6. The expression of TUNEL, 3,3' diaminobenzidine (DAB) chromogen, and methyl green counterstaining in the livers of rats treated with XSS extract and ursodeoxycholic acid at 24 hours is demonstrated. XSS: *Xanthium strumarium* seeds, UDCA: Ursodeoxycholic acid. The positive control group (A) exhibited a high number of TUNEL–positive cells, while the negative control (B), XSS (C), and XSS+UDCA (D) groups demonstrated a low prevalence of TUNEL–positive cells, with a maximum of one cell observed per microscope field under 20 objective

present study demonstrates that cell death in XSS toxicity occurs at the necrotic level, as evidenced by TUNEL staining and classical pathology results.

CONCLUSIONS

In the light of the above mentioned data, it was demonstrated that UDCA treatment can prevent oxidative damage and mitochondrial dysfunction in the kidney and liver tissues of rats exposed to XSS toxicity. This was achieved by decreasing Ca^{2+} amount in mitochondria and inhibiting mPTP opening, which in turn resulted in a reduction in histopathological damage. In light of these findings, UDCA for the treatment of XSS poisoning may be a promising therapeutic option.

Financial Support

This study was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK, 119O859).

Conflicts of interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Zeliha Keskin Alkaç: Writing – original draft preparation, Data curation, Methodology. Fatih Ahmet KORKAK: Methodology. Gürdal DAĞOĞLU: Writing – original draft preparation, Investigation. Yesari ERÖKSÜZ: Histopathology, Immunohistochemistry. Sadettin TANYILDIZI: Writing – original draft preparation, Investigation.

BIBLIOGRAPHIC REFERENCES

[1] Kamboj A, Soluja AK. Phytopharmacological review of *Xanthium strumarium* L. (Cocklebur). Int. J. Green Pharm. [Internet]. 2010; 4(3):129-139. doi: https://doi.org/b7jrzh

[2] Das D, Tangjang S. Bio–stabilization of toxic weeds (*Xanthium strumarium* and *Lantana camara*) implementing mono – and polyculture of *Eisenia fetida* and *Eudrilus eugeniae.* Environ Sci. Pollut. Res. Int. [Internet]. 2024; 31(37):49891-49904. doi: https://doi.org/pgpk

[3] Machado M, Queiroz CRR, Wilson TM, Sousa DER, Castro MB, Saravia A, Lee ST, Armien AG, Barros SS, Riet–Correa F. Endemic *Xanthium strumarium* poisoning in cattle in flooded areas of the Araguari River, Minas Gerais, Brazil. Toxicon [Internet]. 2021; 200:23-29. doi: https://doi.org/pgpm

[4] Sosa S, Capelli A, Corro AC, Dutra F, Santos CGY. Intoxication of dairy cows in Uruguay by ingestion of cocklebur (*Xanthium strumarium*) seeds in sorghum silage. J. Vet. Diagn. Invest. [Internet]. 2025; 37(1):141-144. doi: https://doi.org/pgpn

[5] García–Santos C, Capelli A. Plant and mycotoxin poisonings in ruminants diagnosed in Uruguay. Vet. (Montevideo). [Internet]. 2016 [cited 12 Dec. 2024]; 52(202):28-42. Available in: https://goo.su/uGdzF

[6] Saidi H, Mofid M. Toxic Effect of *Xanthium strumarium* as an Herbal Medicine Preparation. EXCLI J. [Internet]. 2009; 8:115-117. doi: https://doi.org/pgpp

[7] Turgut M, Alhan CC, Gürgöze M, Kurt A, Doğan Y, Tekatli M, Akpolat N, Aygün AD. Carboxyatractyloside poisoning in humans. Ann. Trop. Paediatr. [Internet]. 2005; 25(2):125-34. doi: https://doi.org/fg93nf

[8] Gurley ES, Rahman M, Hossain MJ, Nahar N, Faiz MA, Islam N, Sultana R, Khatun S, Uddin MZ, Haider MS, Islam MS, Ahmed BN, Rahman MW, Mondal UK, Luby SP. Fatal outbreak from consuming Xanthium strumarium seedlings during time of food scarcity in northeastern Bangladesh. Plos One [Internet]. 2010; 5(3):e9756. doi: https://doi.org/fjc5bj

[9] Alves–Figueiredo H, Silva–Platas C, Lozano O, Vázquez–Garza E, Guerrero–Beltrán CE, Zarain–Herzberg A, García–Rivas G. A systematic review of post–translational modifications in the mitochondrial permeability transition pore complex associated with cardiac diseases. Biochim. Biophys. Acta Mol. Basis Dis. [Internet]. 2021; 1867(1):165992. doi: https://doi.org/pgpq

[10] Nikles S, Heuberger H, Hilsdorf E, Schmücker R, Seidenberger R, Bauer R. Influence of Processing on the Content of Toxic Carboxyatractyloside and Artactyloside and the Microbiological Status of *Xanthium sibiricum* Fruits (Ceng'erzi). Planta Med. [Internet]. 2015; 81(12-13):1213-1220. doi: https://doi.org/g6n8gb

[11] Keskin–Alkaç Z, Korkak FA, Dağoğlu G, Eröksüz Y, Tanyıldızı S. Tamoxifen and sodium thiosulfate reduces hepatic and renal damage induced by *Xanthium strumarium* L. Through controlling mitochondrial permeability. Med. Weter. [Internet]. 2025; 81(3):119-127. doi: https://doi.org/pgpr

[12] Kapur A, Ayuso JM, Rehman S, Kumari S, Felder M, Stenerson Z, Skala MC, Beebe D, Barroilhet L, Patankar MS. Oxidative phosphorylation inhibitors inhibit proliferation of endometriosis cells. Reproduction [Internet]. 2023; 165(6):617-628. doi: https://doi.org/pgps

[13] Hofmann AF, Hagey LR. Key discoveries in bile acid chemistry and biology and their clinical applications: history of the last eight decades. J. Lipid. Res. [Internet]. 2014; 55(8):1553-1595. doi: https://doi.org/f6k2hg

[14] Rajesh KG, Suzuki R, Maeda H, Yamamoto M, Yutong X, Sasaguri S. Hydrophilic bile salt ursodeoxycholic acid protects myocardium against reperfusion injury in a PI3K/Akt dependent pathway. J. Mol. Cell. Cardiol. [Internet]. 2005; 39(5):766-776. doi: https://doi.org/ffhm82

[15] Qi H, Shen D, Jiang C, Wang H, Chang M. Ursodeoxycholic acid protects dopaminergic neurons from oxidative stress via regulating mitochondrial function, autophagy, and apoptosis in MPTP/MPP+–induced Parkinson's disease. Neurosci Lett. [Internet]. 2021; 741: 135493. doi: https://doi.org/pgpt

[16] Laurens JB, Bekker LC, Steenkamp V, Stewart MJ. Gas chromatographic–mass spectrometric confirmation of atractyloside in a patient poisoned with *Callilepis laureola*. J. Chromatogr. B. Biomed. Sci. Appl. [Internet]. 2001; 765(2):127-133. doi: https://doi.org/dkfxz8

[17] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. [Internet]. 1979; 95(2):351–358. doi: https://doi.org/bktx4x

[18] Ellman GL. Tissue sulphydryl groups. Arch Biochem. Biophys. [Internet]. 1959; 82(1):70–77. doi: https://doi.org/bz2vt8

[19] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem. [Internet]. 1988; 34(3):497–500. PMID: 3349599. Available in: https://n9.cl/obr0f3

[20] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J. Biol. Chem. [Internet]. 1951; 193(1):265–275. PMID: 14907713. Available in: https://n9.cl/nrvmy

[21] Wang LL, Yu QL, Han L, Ma XL, Song RD, Zhao SN, Zhang WH. Study on the effect of reactive oxygen spesies–mediated oxidative stress on the activation of mitochondrial apoptosis and the tenderness of yak meat. Food Chem. [Internet]. 2018; 244:394-402. doi: https://doi.org/pgpv

[22] Wang LL, Han L, Ma XL. Yu QL, Zhao SN. Effect of mitochondrial apoptotic activation through the mitochondrial membrane permeability transition pore on yak meat tenderness during postmortem aging. Food Chem. [Internet]. 2017; 234:323-331. doi: https://doi.org/g7fw42

[23] Hu ZG, Zhou L, Ding SZ. Effect of aerobic training to exhaustive exercise rat mitochondrial permeability transition pore. J. Shenyang Sport Univ. [Internet]. 2015; 34(3):64-67. Available in: https://goo.su/JVvutD

[24] Steenkamp PA, Harding NM, Van–Heerden FR, van–Wyk BE. Determination of atractyloside in *Callilepis laureola* using solid–phase extraction and liquid chromatography–atmospheric pressure ionisation mass spectrometry. J. Chromatogr A. [Internet]. 2004; 1058(1-2):153-162. doi: https://doi.org/ddx7q2

[25] Alkaç ZK, Korkak FA, Dağoğlu G, İncili CA, Hark BD, Tanyıldızı S. Puerarin mitigates oxidative injuries, opening of mitochondrial permeability transition pores and pathological damage associated with liver and kidney in *Xanthium strumarium*–intoxicated rats. Toxicon [Internet]. 2022; 213:13-22. doi: https://doi.org/pgpx

[26] Koprdova R, Osacka J, Mach M, Kiss A. Acute Impact of Selected Pyridoindole Derivatives on Fos Expression in Different Structures of the Rat Brain. Cell Mol Neurobiol. [Internet]. 2018; 38(1):171-180. doi: https://doi.org/gcwvg6

[27] Dou JP, Wu Q, Fu CH, Zhang DY, Yu J, Meng XW, Liang P. Amplified intracellular Ca 2+ for synergistic anti–tumor therapy of microwave ablation and chemotherapy. J. Nanobiotechnology [Internet]. 2019; 17:1-17. doi: https://doi.org/gp5dqc

[28] Nolfi–Donegan D, Braganza A, Shiva S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. Redox Biol. [Internet]. 2020; 37:101674. doi: https://doi.org/gmxqv7

[29] Wang Y, Han T, Xue M, Han P, Zhang QY, Huang BK, Zhang H, Ming QL, Peng W, Qin LP. Hepatotoxicity of kaurene glycosides from *Xanthium strumraium* L. fruits in mice. Pharmazie. [Internet]. 2011; 66(6):445-449. doi: https://doi.org/pgpz

[30] Liu R, Shi D, Zhang J, Li X, Han X, Yao X, Fang J. Xanthatin Promotes Apoptosis via Inhibiting Thioredoxin Reductase and Eliciting Oxidative Stress. Mol. Pharm. [Internet]. 2018; 15(8):3285-3296. doi: https://doi.org/gdsvwj

[31] Atlante A, Valenti D, Latina V, Amadoro G. Dysfunction of Mitochondria in Alzheimer's Disease: ANT and VDAC Interact with Toxic Proteins and Aid to Determine the Fate of Brain Cells. Int. J. Mol. Sci. [Internet]. 2022; 23(14):7722. doi: https://doi.org/pgp3

[32] Nirody JA, Budin I, Rangamani P. ATP synthase: Evolution, energetics, and membrane interactions. J. Gen. Physiol. [Internet]. 2020; 152(11):e201912475. doi: https://doi.org/g89s3m

[33] Campanella M, Parker N, Tan CH, Hall AM, Duchen MR. IF1: setting the pace of the F1F0-ATP synthase. Trends. Biochem. Sci. [Internet]. 2009; 34(7):343-350. doi: https://doi.org/cpcpn3

[34] Grover GJ, Atwal KS, Sleph PG, Wang FL, Monshizadegan H, Monticello T, Green DW. Excessive ATP hydrolysis in ischemic myocardium by mitochondrial F1F0-ATPase; effect of selective pharmacological inhibition of mitochondrial ATPase hydrolase activity. Am. J. Physiol. Heart. Circ. Physiol. [Internet]. 2004; 287(4):H1747-H1755. doi: https://doi.org/d98427

[35] Koc S, Aktas A, Sahin B, Ozer H, Zararsiz GE. Protective effect of ursodeoxycholic acid and resveratrol against tacrolimus induced hepatotoxicity. Biotech. Histochem. [Internet]. 2023; 98(7):471-478. doi: https://doi.org/pgp4

[36] Simental–Mendía M, Sánchez–García A, Simental–Mendía LE. Effect of ursodeoxycholic acid on liver markers: A systematic review and meta–analysis of randomized placebo–controlled clinical trials. Br. J. Clin. Pharmacol. [Internet]. 2020; 86(8):1476-1488. doi: https://doi.org/pgp5

[37] Rajagopala SV, Singh H, Yu Y, Zabokrtsky KB, Torralba MG, Moncera KJ, Pieper R, Sender L, Nelson KE. Persistent gut microbial dysbiosis in children with acute lymphoblastic leukemia (ALL) during chemotherapy. Microb. Ecol. [Internet]. 2020; 79:1034-1043. doi: https://doi.org/gmwp4f

[38] Qi H, Shen D, Jiang C, Wang H, Chang M. Ursodeoxycholic acid protects dopaminergic neurons from oxidative stress via regulating mitochondrial function, autophagy, and apoptosis in MPTP/MPP$^+$–induced Parkinson's disease. Neurosci. Lett. [Internet]. 2021; 741:135493. doi: https://doi.org/pgpt

[39] Ali FEM, Hassanein EHM, Bakr AG, El–Shoura EAM, El–Gamal DA, Mahmoud AR, Abd–Elhamid TH. Ursodeoxycholic acid abrogates gentamicin–induced hepatotoxicity in rats: Role of NF–KB–p65/TNF–a, Bax/Bcl–xl/Caspase-3, and eNOS/iNOS pathways. Life Sci. 2020; 254:117760. doi: https://doi.org/gt5kh7

[40] Xue LM, Zhang QY, Han P, Jiang YP, Yan RD, Wang Y, Rahman K, Jia M, Han T, Qin LP. Hepatotoxic constituents and toxicological mechanism of *Xanthium strumarium* L. fruits. J. Ethnopharmacol. [Internet]. 2014; 152(2):272-282. doi: https://doi.org/f5wjfd

[41] Deng Z, He M, Hu H, Zhang W, Zhang Y, Ge Y, Ma T, Wu J, Li L, Sun M, An S, Li J, Huang Q, Gong S, Zhang J, Chen Z, Zeng Z. Melatonin attenuates sepsis–induced acute kidney injury by promoting mitophagy through SIRT3-mediated TFAM deacetylation. Autophagy [Internet]. 2024; 20(1):151-165. doi: https://doi.org/gsqgnt

[42] Li L, Han W, Gu Y, Qiu S, Lu Q, Jin J, Luo J, Hu X. Honokiol induces a necrotic cell death through the mitochondrial permeability transition pore. Cancer Res. [Internet]. 2007; 67(10):4894-4903. doi: https://doi.org/c7nj9s

[43] Xu H, Sun Y, Zhang Y, Wang W, Dan J, Yao J, Chen H, Tian F, Sun X, Guo S, Tian Z, Tian Y. Protoporphyrin IX induces a necrotic cell death in human THP-1 macrophages through activation of reactive oxygen species/c–Jun N–terminal protein kinase pathway and opening of mitochondrial permeability transition pore. Cell. Physiol. Biochem. [Internet]. 2014; 34(6):1835-1848. doi: https://doi.org/f6vtq4