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TAUROURSODEOXYCHOLIC ACID MITIGATES CYCLOPHOSPHAMIDE-INDUCED HEPATO-RENAL TOXICITY IN RATS: ATTENUATION OF OXIDATIVE STRESS AND INFLAMMATION

AMIRA SAMIR [1]; ASHRAF EL KOMY [2]; ENAS FARAG [3] AND AHMED ABDEEN [4]

[1] Pharmacist at Benha University Hospital, Qalyubia, Egypt.
[2] Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Toukh, Qalyubia, Egypt.
[3] Deputy of AHRI for regional laboratories. AHRI. ARC, Cairo, Egypt.
[4] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Toukh, Qalyubia, Egypt.

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ABSTRACT

Cyclophosphamide (CP) is a powerful anticancer drug, but its use is limited by side effects like liver and kidney damage. Tauroursodeoxycholic acid (TUDCA) is a natural bile acid with antioxidant, anti-apoptotic, and anti-inflammatory activities. This study investigated whether TUDCA could protect against CP-induced liver and kidney damage in rats. Male rats (n=24) were divided into four groups (n=6/group): control, TUDCA (300 mg/kg/day orally for 14 days), CP (single 200 mg/kg i.p. injection on day 10), and TUDCA + CP (TUDCA for 14 days followed by CP injection on day 10). After 4 days, blood and tissue samples were collected for analysis of kidney and liver function (urea, creatinine, AST, ALT, ALP, total bilirubin, total protein, and albumin), blood cell counts (WBCs, RBCs, HB, and platelets), lipid profile (triglycerides, cholesterol, and HDL), glucose, oxidative stress markers (MDA, CAT, GSH), and histological immunohistochemical examinations. CP administration significantly increased markers of kidney (urea, creatinine) and liver (AST, ALT, ALP) damage. CP also caused abnormal blood chemistry (increased triglycerides, cholesterol, total bilirubin, and glucose) and decreased HDL, total protein, albumin, WBCs, RBCs, HB, and platelets. CP increased oxidative stress markers (MDA) and decreased antioxidant enzymes (CAT, GSH) in the kidney and liver. Histological and immunohistochemical analysis revealed severe damage in CP-treated animals, including kidney atrophy and liver disorganization. TUDCA pretreatment exhibited protective effects against CP-induced hepatorenal toxicity, alleviating oxidative stress and inflammation, likely due to its antioxidant properties. However, further studies are required to validate its potential therapeutic use in human conditions.

Keywords: Cyclophosphamide; Liver damage; Oxidative stress; Renal toxicity; Tauroursodeoxycholic acid.

Corresponding author: Amira Samir and Ahmed Abdeen
E-mail address: amirasamir01@yahoo.com; ahmed.abdeen@fvtm.bu.edu.eg
Present address: [1]Pharmacist at Benha University Hospital, Qalyubia, Egypt;

INTRODUCTION

CP is a cytotoxic alkylating agent belonging to the oxazaphosphorine class and derived from nitrogen mustard. CP exerts its therapeutic effects through a variety of mechanisms, including

inhibition of angiogenesis and modulation of the immune system. This broad-spectrum chemotherapeutic agent is indicated for the treatment of numerous cancer types, such as leukaemia, lymphoma, ovarian, breast, and lung cancers (Hu *et al*., 2022; Mishra *et al*., 2022).

In spite of being a potential chemotherapeutic agent, CP's clinical application is often limited due to its severe side effects, such as neurotoxicity, cardiotoxicity, nephrotoxicity, hepato-toxicity, immunotoxicity, genotoxicity, and bone marrow suppression (Caglayan, 2019).

More than any other organs, the liver and kidney play a major role in material detoxification through the metabolism and excretion of chemical substances. Because of this, the toxicity of chemotherapy medications can cause serious kidney and liver damage as well as degenerative changes (Un *et al*., 2020).

Nephrotoxicity is a major concern associated with CP use (Ghareeb *et al*., 2019). Hepatotoxicity is a significant adverse effect of CP resulting from its hepatic metabolism. The drug is converted by cytochrome P450 enzymes into active metabolites, including phosphoramide mustard and acrolein (Mishra *et al*., 2022). While phosphoramide mustard contributes to CP's anticancer properties, acrolein is primarily responsible for its toxic effects, including liver damage that can compromise organ function and even cause organ failure (Madubogwu *et al*., 2022).
Given the potential for severe organ damage caused by CP, there is a clear need for protective measures. Administering antioxidants before or during CP treatment may help to mitigate these adverse effects, particularly on the liver, kidneys, and cardiovascular system.

Bile acids are one newly developed treatment for several cellular programmed death pathways (Grant & DeMorrow, 2020). Among the most hydrophilic bile acids are TUDCA and ursodeoxycholic acid (UDCA) (Khalaf *et al*., 2022). Bile acids have the benefit of being administered via oral, subcutaneous, and intravenous methods of administration when used therapeutically (Kusaczuk, 2019).

"Chemical chaperones" are a class of pharmacological drugs with the potential to be helpful in the treatment of endoplasmic reticulum stress. TUDCA is one of these compounds that are recognized for their chaperoning property (Kusaczuk, 2019). It is a naturally occurring hydrophilic tertiary bile acid that is composed of taurine amino acid conjugated with UDCA (Hou *et al*., 2021).

TUDCA has garnered significant attention due to its cytoprotective, neuroprotective, and anti-apoptotic properties (Fu *et al*., 2021). TUDCA exerts its protective effects through multiple mechanisms, such as stabilizing mitochondrial membranes, reducing endoplasmic reticulum (ER) stress, and stimulating antioxidant pathways. The reduction of ER stress and pro-inflammatory cytokine release plays a vital role in preventing organ toxicity caused by various chemicals, including CP cancers (Hou *et al*., 2017; Sabat *et al*., 2021).

Given its broad spectrum of therapeutic potential, TUDCA has been investigated for various diseases. The US Food and Drug Administration (FDA) has approved TUDCA for liver disorders like cholestasis, cirrhosis, and hepatitis (Hou *et al*., 2021). Additionally, TUDCA has demonstrated renal protective effects in several studies (Angelico *et al*., 1995; De Miguel *et al*., 2019; Kusaczuk, 2019).

TUDCA was chosen for this study due to its well-documented cytoprotective properties, including its antioxidant, anti-inflammatory, and anti-apoptotic effects. It

is known to reduce ER stress, which plays a crucial role in organ toxicity. Since CP induces hepato-renal damage through oxidative stress and inflammation, TUDCA was selected as a potential protective agent to counteract these harmful effects and mitigate CP-induced toxicity in liver and kidney tissues.

This research aimed to assess TUDCA's efficacy in mitigating CP-induced nephron-toxicity and hepatotoxicity by evaluating serum biomarkers, histopathological changes, and oxidative stress levels.

MATERIALS AND METHODS

1. Drugs:

CP (Endoxan® 1gm) was obtained from Baxter Oncology Chemical Co. (Egypt) and dissolved in physiological saline for intraperitoneal (IP) injection. TUDCA used in this study, at a concentration of 500 mg, was obtained from Doubla Wood Co., PA, USA, and dissolved in normal saline.

2. Experimental Animals:

Twenty-four adult male Wistar Albino rats weighing 160–200 g and aged 8 weeks old were obtained from the Laboratory Animal Center, Benha University. Animals were housed under standard conditions for 2 weeks at a temperature of (23 ± 2)°C and provided with a commercial diet with unhindered access to water. The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Benha University (BUFVTM 04-04-24). After 2 weeks of acclimatization, all animals were randomly divided into four groups (6 each):

- **Group I (Control):** Received oral saline for 14 consecutive days.
- Group II (TUDCA): Received oral TUDCA (300 mg/kg/day) for 14 consecutive days. This dose is according to **Rivard *et al*. (2007)**.
- **Group III (CP):** Received a single IP injection of CP (200 mg/kg) on day 10 **(Temel *et al*., 2020)**.
- **Group IV (TUDCA+CP):** Received oral TUDCA (300 mg/kg/day) for 10 days, followed by an IP single dose of CP (200 mg/kg) on day 10 and continued TUDCA for the remaining 4 days of the study.

3. Sample collection and biochemical analysis:

At the end of the trial, the rats were sacrificed under the conventional protocol of isoflurane (Isoflurane-Sedico®) inhalation anaesthesia produced by Sedico Company (Abdelnaby *et al*., 2022). Next, blood samples were immediately obtained from the inferior vena cava, centrifuged at 2,000g for 15 min for serum separation, and stored at −20°C for biochemical bioassay. The liver and kidney tissues were swiftly dissected and permeated in ice-cold saline to remove blood clots. After that, each tissue sample was dissected into two halves, one of which was kept in a 10% buffered formalin solution for further histological and immunohistochemical investigation, while the other portion was stored at -20°C for subsequent evaluation of oxidative cascade markers.

Serum biochemical markers, including ALT, AST, ALP, total bilirubin, cholesterol, high-density lipoprotein, triglycerides, total proteins, glucose, urea, albumin, and creatinine, were assessed using commercial kits purchased from Bio Diagnostic Company (Giza, Egypt). AST (Cat. No. AS1061), ALT (Cat. No. AL1031), ALP (Cat. No. AP1021), urea (Cat. No. UR2110), creatinine (Cat. No. CR1250), total protein (Cat. No. TP2020), albumin (Cat. No. AB1010), total bilirubin (Cat. No. BR1111), triglycerides, cholesterol (Cat. No. TG117249), high-density lipoprotein (Cat. No. MG266001), and

glucose (Cat. No. GLU109240) were performed according to the manufacturer's instructions to evaluate liver and kidney function (Ukpo *et al*., 2013). Moreover, blood samples were collected into EDTA tubes on day 14 and analyzed for RBCs, haemoglobin, platelets, and WBCs.

4. Tissue homogenization and oxidative stress assessment:

Tissue samples were homogenized in a heparin-containing PBS solution using a DAIHAN Scientific CO., Ltd. homogenizer (HG-15A) and centrifuged to remove cellular debris. The supernatant was collected and stored at -80°C for subsequent analysis. Oxidative stress parameters, including reduced glutathione (GSH), catalase (CAT), and malondialdehyde (MDA) levels, were evaluated in liver and kidney tissue homogenate (Sedlak & Lindsay, 1968; Ohkawa *et al*., 1979; Aebi, 1984) using commercial kits of GSH (Cat. No. GR 2511), MDA (Cat. No. MD 2529), and CAT (Cat. No. 2533) that were supplied by the Laboratory Bio diagnostic Company according to established protocols.

5. Histological Examination:

Tissue samples were sliced to 3-4 mm thick and fixed in 10% neutral-buffered formalin (10% NBF) for 24 h, embedded in paraffin, sectioned at 4-6 µm thickness, and stained with hematoxylin and eosin (H and E) (Bancroft & Stevens, 2016). Histological examination was performed using a Leica microscope (CH9435 Hee56rbrugg).

6. Immunohistochemistry and immunoscoring protocol:

Paraffin-embedded tissue sections were deparaffinized, rehydrated, and subjected to heat-induced antigen retrieval at 120 ℃ for 5 min. Immunostaining was performed using the avidin-biotin-peroxidase complex (ABC) method. Subsequently, tissue sections were subjected to antigen retrieval by heating in a citric acid solution for 5 min. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide (H_2O_2) for 5 min, followed by three rinses in phosphate-buffered saline (PBS). To minimize nonspecific binding, sections were incubated with 5% bovine serum albumin (BSA) blocking solution for 20 min. Sections were then incubated overnight at 4°C with a rabbit anti-IL-1β primary antibody (clone RM1009, Abcam cat# ab283818, 1:500 dilution). After washing, the ABC reagent (Vector Laboratories) was applied, followed by diaminobenzidine (DAB) substrate for peroxidase visualization. Negative controls were processed using non-immune serum instead of the primary antibody. Stained sections were imaged under a Leica microscope (CH9435 Heerbrugg). Quantitative analysis of IL-1β expression was conducted by calculating the area percentage of DAB-positive staining in three randomly selected high-power fields (×400 magnification) per section using Leica QWin 500 image analysis software.

7. Statistical Analysis:

Data were analyzed using one-way ANOVA followed by Duncan's (1995) as a post hoc test to compare differences between groups. Statistical significance was set at $P < 0.05$. All statistical analyses were conducted using SPSS (Version 21).

RESULTS

1. Biochemical and haematological assays:

CP intoxication significantly elevated serum levels of AST, ALT, ALP, and bilirubin ($P<0.05$) compared to the control group. Conversely, serum albumin and total protein levels were markedly decreased ($P<0.05$) in CP-treated rats. Pretreatment with TUDCA significantly attenuated the CP-induced increase in AST, ALT, ALP, and bilirubin levels ($P<0.05$). Furthermore, TUDCA treatment significantly reversed the CP-induced decrease in serum

albumin and total protein levels (P<0.05) (Table 1).

CP intoxication significantly elevated serum urea and creatinine levels compared to the control group (P<0.05), indicating kidney damage. Pretreatment with TUDCA significantly reduced these elevated levels ($p < 0.05$), suggesting a protective effect on kidney function (Table 1).

Table 1: Impact of serum biochemical tests following CP and/or TUDCA treatment.

	Creatinine (mg/dL)	Urea (mg/dL)	ALT (U/L)	AST (U/L)	ALP (U/L)	BIT (mg/dL)	ALB (mg/dL)	TPRO (mg/dL)
Control	0.37±0.02^{c}	21.75±1.69^{c}	16.30±1.54^{c}	32.08±1.08^{c}	105.03±2.09^{c}	0.20±0.02^{c}	4.03±0.04^{a}	6.25±0.31^{a}
TUDCA	0.40±0.04^{bc}	22.42±0.49^{c}	15.77±0.58^{c}	33.46±1.39^{c}	104.12±1.44^{c}	0.22±0.01^{c}	4.15±0.33^{a}	6.40±0.03^{a}
CP	1.46±0.18^{a}	53.00±3.81^{a}	44.74±0.80^{a}	106.48±6.58^{a}	295.70±14.55^{a}	0.53±0.02^{a}	1.90±0.13^{c}	3.55±0.13^{c}
CP + TUDCA	0.75±0.05^{b}	36.69±0.71^{b}	31.14±1.38^{b}	69.71±2.91^{b}	136.83±7.60^{b}	0.36±0.00^{b}	3.47±0.02^{b}	4.97±0.02^{b}

CP, cyclophosphamide; TUDCA, tauroursodeoxycholic acid; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BIT, total bilirubin; ALB, albumin; TPRO, total protein. Data are presented as mean ± SE (n = 6). Different small letters indicate a significant difference between groups at P<0.05.

CP significantly increased serum cholesterol and triglyceride levels while decreasing HDL-cholesterol compared to the control group (P<0.05). Treatment with TUDCA reversed these lipid profile abnormalities, significantly reduced cholesterol and triglyceride levels and increased HDL-cholesterol (P<0.05) (Figure 1).

Serum glucose levels were significantly different among the treatment groups (P<0.05). The TUDCA-treated group exhibited higher glucose levels compared to the control group, while both CP-treated groups showed lower glucose levels (Figure 1).

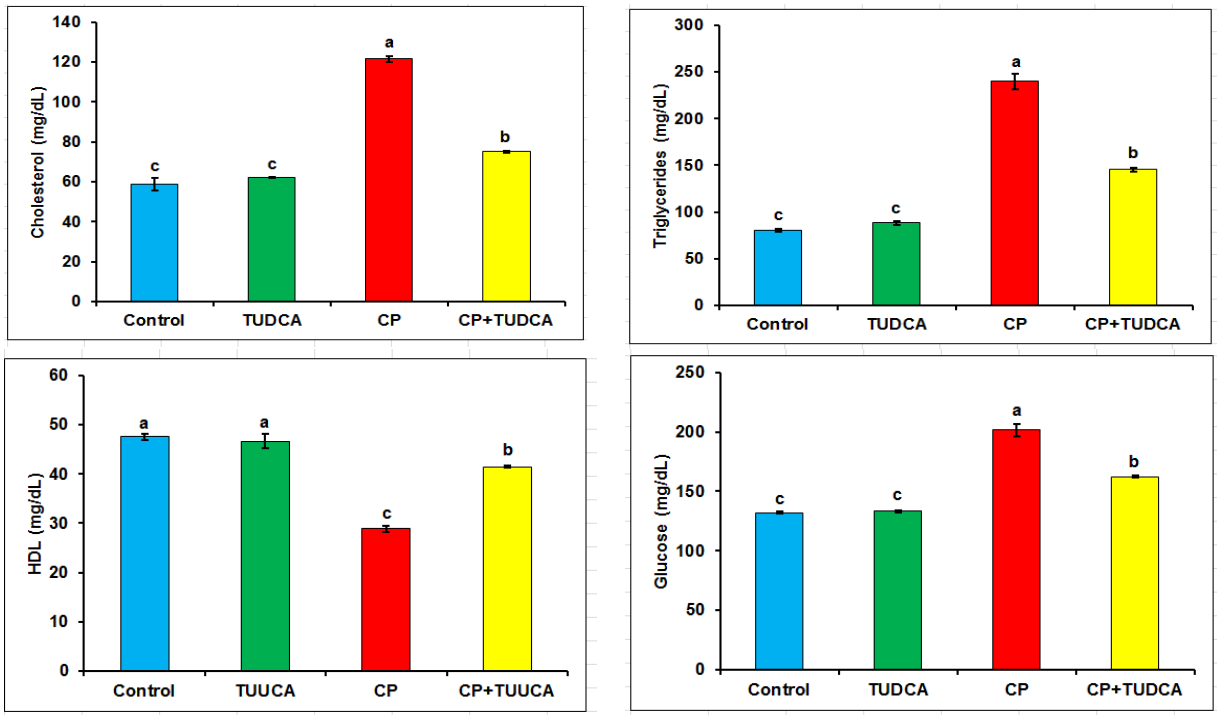


Figure 1: Bar plot panel of serum biochemical tests following CP and/or TUDCA treatment. CP, cyclophosphamide; TUDCA, tauroursodeoxycholic acid; HDL, high-density lipoprotein. Data are exhibited as mean ± SE (n = 6). Different small letters indicate a significant difference between groups at P < 0.05.

Hematological analysis revealed a significant decrease (P<0.05) in white blood cells (WBCs), red blood cells (RBCs), platelets (PLTs), and haemoglobin (HGB) levels in CP-

treated rats compared to the control group. Treatment with TUDCA significantly ameliorated these haematological alterations, increasing WBCs, RBCs, PLTs, and HGB levels compared to the CP-treated group (Table 2).

Table 2: Impact of haematological tests following CP and/or TUDCA treatment.

	WBCs (103/µL)	RBCs (106/µL)	HGB (g/dL)	PLTs (103/µL)
Control	8.40±0.35[a]	6.07±0.05[a]	14.62±0.44[a]	851.17±26.90[a]
TUDCA	8.18±0.20[a]	6.30±0.08[a]	14.18±0.25[a]	798.00±24.75[a]
CP	2.54±0.01[c]	2.20±0.34[c]	5.22±0.81[c]	114.60±2.46[c]
CP+ TUDCA	6.14±0.04[b]	5.11±0.12[b]	10.63±0.24[b]	389.17±13.30[b]

CP, cyclophosphamide; TUDCA, tauroursodeoxycholic acid; HGB, haemoglobin; WBCs, white blood cells; RBCs, red blood cells; PLTs, platelets. Data are presented as mean ± SE (n = 6). Different small letters indicate a significant difference between groups at P<0.05.

2. Cellular antioxidants and peroxidation indices:

CP induced significant oxidative stress in both liver and kidney tissues, as evidenced by decreased CAT and GSH levels and increased MDA levels compared to the control group (P<0.05). Treatment with TUDCA significantly attenuated CP-induced oxidative stress by increasing CAT and GSH levels while decreasing MDA levels (P<0.05) (Figure 2).

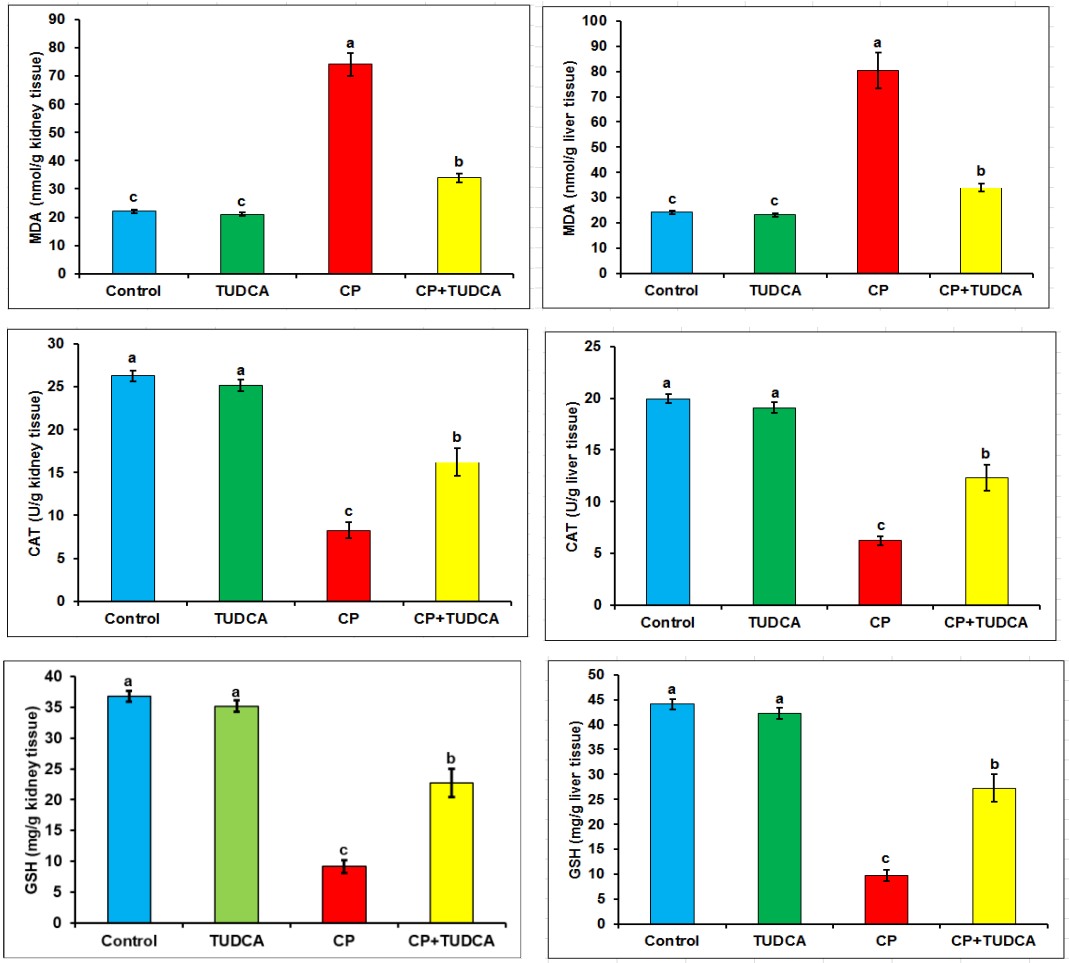


Figure 2: Bar plot panel of oxidant/antioxidant indices following CP and/or TUDCA treatment in liver and kidney tissues. CP, cyclophosphamide; TUDCA, tauroursodeoxycholic acid; CAT, catalase; GSH, reduced glutathione; MDA, malondialdehyde. Data are exhibited as mean ± SE (n = 6). Different small letters indicate a significant difference between groups at P<0.05

3. Hepato-renal pathological changes of TUDCA on CP-exposed rats:

Histopathological observations of the present work of the hepatic tissue of CP-

injected mice show congestion and hyalinization of the central vein, loss of hepatic cord organization, hypertrophy and hydropic degeneration of hepatocytes, other hepatocytes appearing with deep acidophilic cytoplasm and deep basophilic apoptotic nuclei, interstitial oedema, vacuolation between hepatic cords, and atrophy and congestion along hepatic sinusoids. Examination of the livers of the CP-injected mice that were treated with TUDCA showed obvious improvement along hepatic tissue with intact central vein endothelial lining; some hepatic cords existed either with normal organization and lined with normal vesicular hepatocytes or with high vacuolation (Figure 3).

Control and TUDCA groups (Figure 3A, B): Normal hepatic architecture with intact central vein, well-organized hepatic cords, and normal hepatocytes. CP group (Figure 3C): Severe liver damage characterized by central vein congestion, loss of hepatic cord structure, hepatocyte degeneration, and inflammatory cell infiltration. CP + TUDCA group (Figure 3D): Partial recovery of liver tissue with reduced inflammation and some restoration of hepatic cord architecture. However, persistent hepatocyte damage is evident.

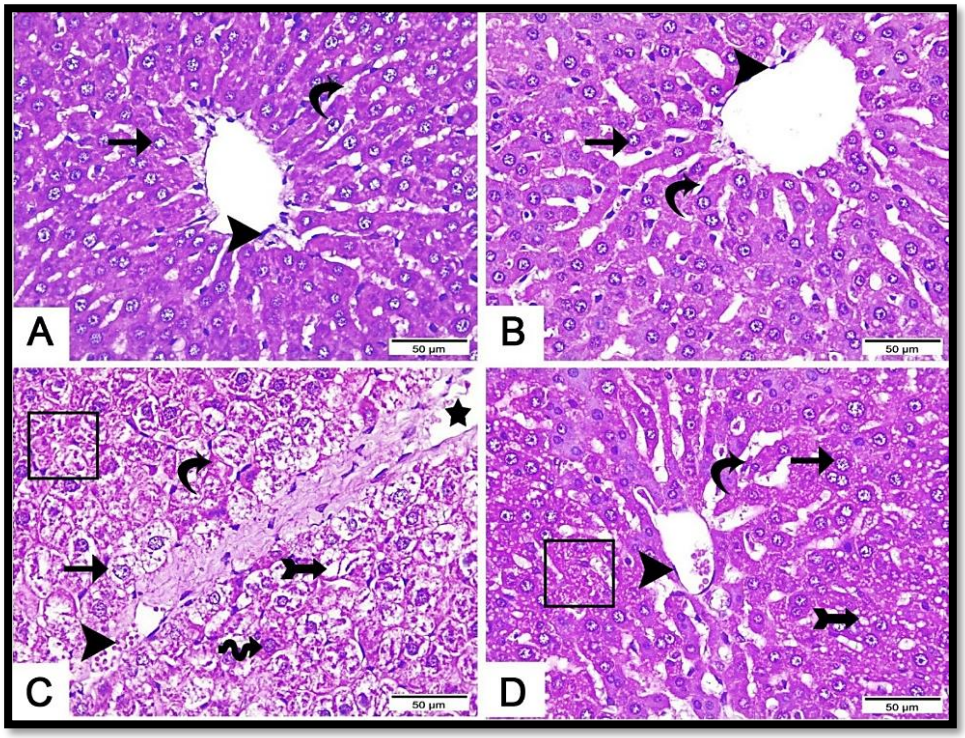


Figure 3: Histopathology of liver tissue in control, CP, TUDCA, and CP+TUDCA-treated groups**.**

Section from control group (A) & TUDCA group (B) highlighting the standard structure of the central vein area with intact central vein endothelial lining (arrowhead); hepatic cords presented with their regular organization (arrow). Hepatic sinusoids are observed between hepatic cords with their usual structure (curvy arrow). Section from CP group (C) displaying severe degenerative changes along hepatic tissue noticed with congestion & hyalinization of central vein (arrowhead), loss of hepatic cord organization (rectangle), hypertrophy & hydropic degeneration of hepatocytes (arrow), other hepatocytes appeared with deep acidophilic cytoplasm & deep basophilic apoptotic nuclei (wave arrow), interstitial edema (star), vacuolation between hepatic cords is seen (arrow with tail), with atrophy & congestion along hepatic sinusoids (curvy arrow). Section

from CP & TUDCA-treated group (D) showing noticeable improvement along hepatic tissue with intact central vein endothelial lining (arrowhead); some hepatic cords existed either with normal organization (arrow) or with high vacuolation (arrow with tail). While other hepatocytes still seemed degenerated and lost their organization (rectangle). Also, dilatation & congestion along hepatic sinusoids (curvy arrow). (H and E stain, magnification power = x400 & scale bar = 50 µm).

The results revealed that mice injected with CP induced histological alterations in the kidney tissues, marking severe injury along the renal cortex area, evidenced by atrophy, vacuolation, apoptotic glomerulus lining cells, and degenerated Bowman's capsule. Some renal tubules appeared with severe degeneration, some existed with epithelial desquamation, and others were lined with deep basophilic apoptotic cells and surrounded by a hollow area. Also, some tubules appeared dilated; additionally, some tubules' lumens were seen with hyaline casts. Moreover, interstitial oedema was detected with dispersion between renal tubules.

While the kidney of the mice treated with TUDCA before CP showed noticeable development along the renal cortex as some renal corpuscles emerged with their normal structure, others still appeared with vacuolation and a hyalinized Bowman's capsule. Some renal tubules were observed normal in look; however, some of them appeared with collapsed lumen and epithelial desquamation, and some were lined with some deep basophilic apoptotic nuclei, and a few of them still appeared with loss of their organization and severe degeneration (Figure 4). Control and TUDCA groups (Figure 4A, B): Normal renal architecture with intact renal corpuscles, proximal convoluted tubules (PCT), and distal convoluted tubules (DCT). CP group (Figure 4C): Severe renal damage characterized by glomerular atrophy, tubular degeneration, necrosis, and interstitial oedema. CP + TUDCA group (Figure 4D): Partial recovery of renal tissue with some restoration of normal structures. However, persistent tubular damage is evident.

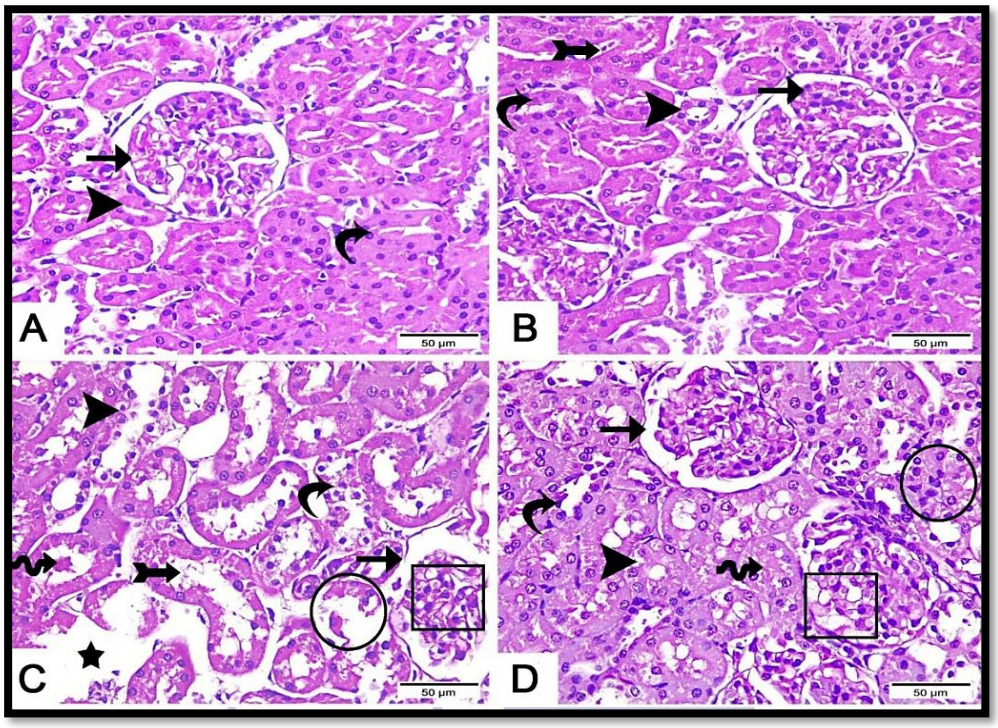


Figure 4: Histopathology of kidney tissue in control, CP, TUDCA, and CP+TUDCA-treated groups.

Sections from control group (A) & TUDCA group (B) revealing the standard structure of the renal cortex area with normal renal corpuscle assembling as Bowman's capsule & glomerulus (arrow), proximal convoluted tubule (PCT) (arrowhead), & distal convoluted tubule (DCT) (curvy arrow). Notice the usual extent of renal tubule lumen (arrow with tail). Section from CP group (C) displaying severe injury along the renal cortex area, evidenced by atrophy, vacuolation, and apoptotic glomerulus lining cells (rectangle) and degenerated Bowman's capsule (arrow). Some renal tubules appeared with severe degeneration (circle), some existed with epithelial desquamation (curvy arrow), & others were lined with deep basophilic apoptotic cells & surrounded with hallow (arrowhead); also, some tubules appeared dilated (arrow with tail), and additionally, some tubules' lumen were seen with hyaline cast (wave arrow). Moreover, interstitial oedema was detected with dispersion between renal tubules (star). Section from CP & TUDCA-treated group (D) highlighting noticeable development along the renal cortex as some renal corpuscles emerged with their normal structure (arrow), while others still appeared with vacuolation & hyalinized Bowman's capsule (rectangle). Some renal tubules observed in normal look (arrowhead), however, some of them appeared with collapsed lumen & epithelial desquamation (wave arrow), also some lined with some deep basophilic apoptotic nuclei (curvy arrow), & a few of them still appeared with loss of their organization & severe degeneration (circle). (H and E stain, magnification power = x400 & scale bar = 50 μm).

4. Hepato-renal effect of TUDCA on IL-1β expression of CP-exposed rats:

CP induced a significant increase in interleukin-1β (IL-1β) expression in liver tissue compared to both the control and TUDCA-treated groups (P≤0.05). While TUDCA treatment led to a reduction in IL-1β expression compared to the CP group, it remained elevated compared to the control (Figure 5). Control and TUDCA groups (Figure 5A, B): Low levels of interleukin-1β expression in hepatocytes. CP group (Figure 5C): Significant increase in IL-1β expression in hepatocytes compared to control and TUDCA groups (P≤0.05). CP + TUDCA group (Figure 5D): Moderate IL-1β expression in hepatocytes, significantly lower than the CP group (P≤0.05) but higher than the control and TUDCA groups. (Figure 5E): IL-1β expression, as assessed by area percentage, was significantly increased in the liver tissues of CP-treated rats compared to the control group (P≤0.05). Pretreatment with TUDCA significantly reduced IL-1β expression compared to the CP-treated group (P≤0.05).

Sections from the negative control group (A) & TUDCA group (B) showing scarce positive cytoplasmic expression of IL-1β along hepatocytes (arrow) with non-significant difference (P≥0.05) between them. Section from CP group (C) demonstrating the highest positive cytoplasmic expression of IL-1β along hepatocytes (arrow) with a significant difference (P≤0.05) from the negative control group & TUDCA group. Section from CP & TUDCA group (D) exhibiting moderate positive cytoplasmic expression of IL-1β along hepatocytes (arrow) with significant difference (P≤0.05) from other groups. (E) IL-1β expression, as assessed by area percentage, was significantly increased in the liver tissues of CP-treated rats compared to the control group (P≤0.05). Pretreatment with TUDCA significantly reduced IL-1β expression compared to the CP-treated group (P≤0.05). (Magnification power = X400, scale bar = 50μm).

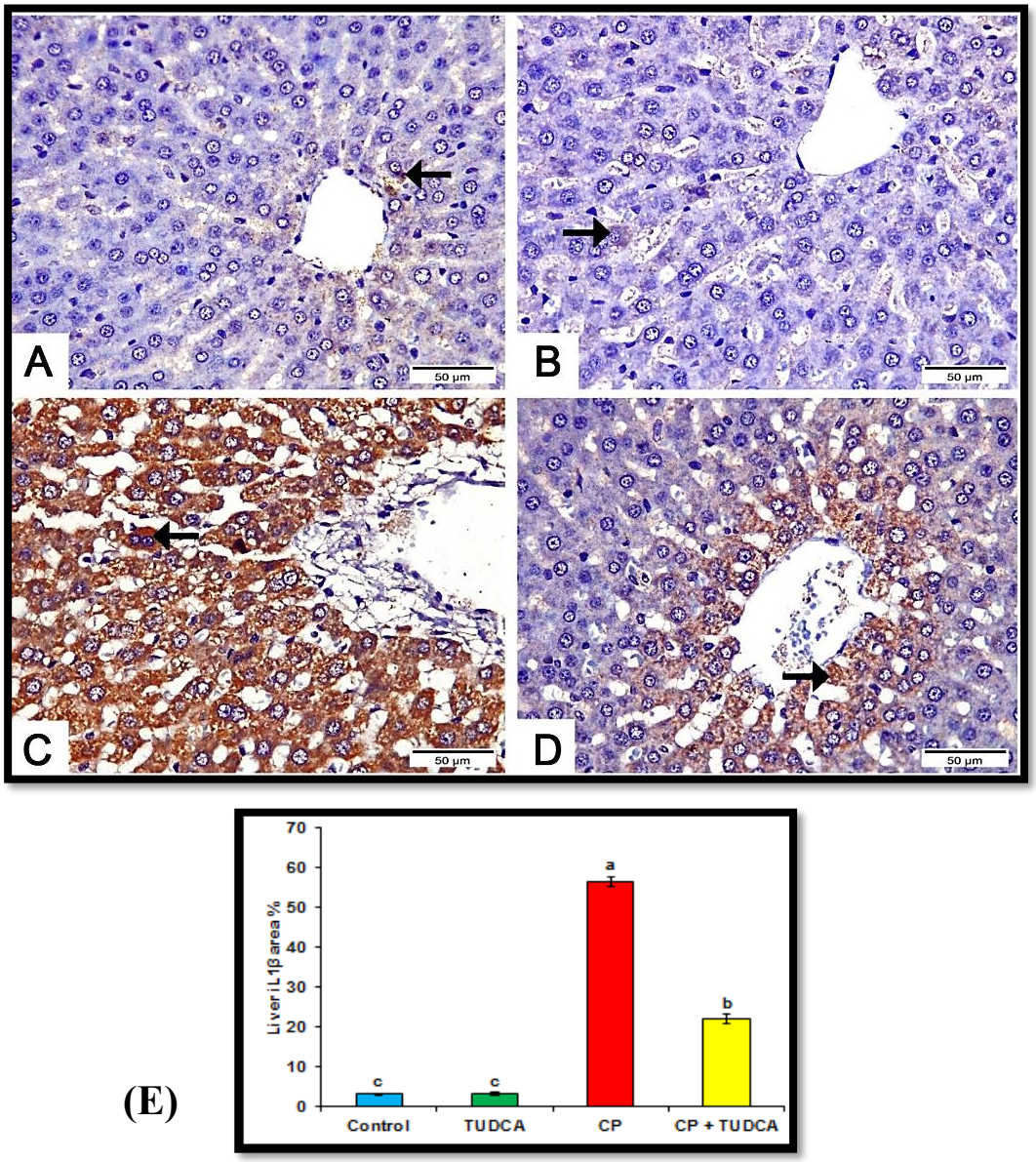
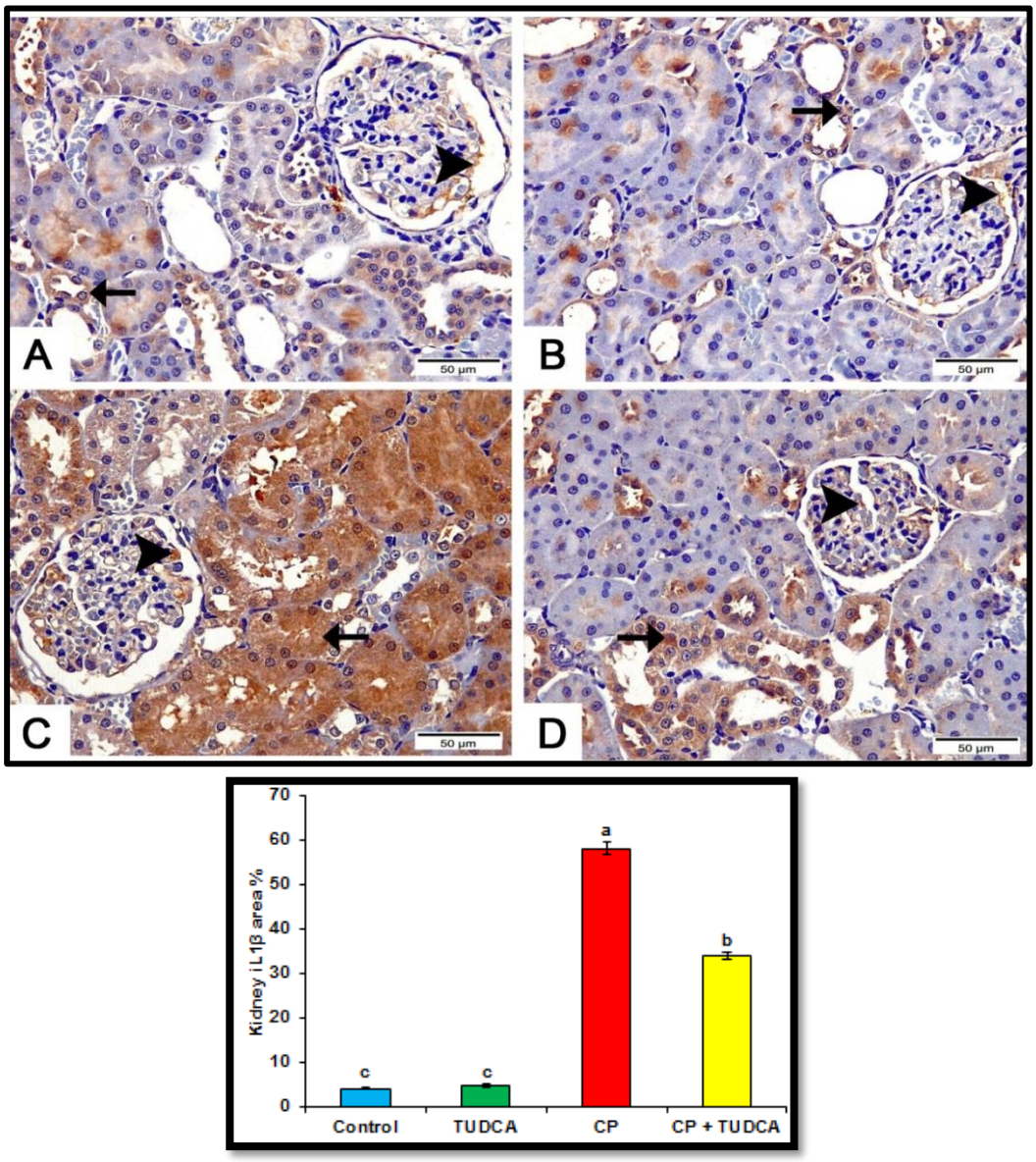


Figure 5: Effect of the dose-dependent manner of TUDCA supplementation on IL-1β expression following CP-intoxication in the liver.

CP induced a significant increase in interleukin-1β expression in both renal tubules and glomeruli compared to both the control and TUDCA-treated groups (P≤0.05). TUDCA treatment led to a reduction in interleukin-1β expression compared to the CP group, although levels remained elevated compared to the control (Figure 6). Control and TUDCA groups (Figure 6A, B): Low levels of IL-1β expression in renal tubules and glomeruli. CP group (Figure 6C): Significant increase in IL-1β expression in both renal tubules and glomeruli compared to control and TUDCA groups (P≤0.05). CP + TUDCA group (Figure 6D): Moderate IL-1β expression in renal tubules and glomeruli, significantly lower than the CP group but higher than the control and TUDCA groups (P≤0.05). (Figure 6E): IL-1β expression, as assessed by area percentage, was significantly increased in the kidney tissues of CP-treated rats compared to the control group (P≤0.05). Pretreatment with TUDCA significantly reduced IL-1β expression compared to the CP-treated group (P≤0.05).



(E)

Figure 6: Effect of the dose-dependent manner of TUDCA supplementation on IL-1β expression following CP-intoxication in the kidney.

Sections from the negative control group (A) & TUDCA group (B) marking scarce positive cytoplasmic expression of IL-1β along renal tubules (arrow), & the glomerulus (arrowhead), with non-significant difference (P≥0.05) between them. Section from CP group (C) revealing the highest positive cytoplasmic expression of IL-1β along renal tubules (arrow) as well as the glomerulus (arrowhead), with a significant difference (P≤0.05) from the negative control group & TUDCA group. Section from CP & TUDCA group (D) showing moderate positive cytoplasmic expression of IL-1β along renal tubules (arrow), & the glomerulus (arrowhead) with significant difference (P≤0.05) from other groups. (E) IL-1β expression, as assessed by area percentage, was significantly increased in the kidney tissues of CP-treated rats compared to the control group (P≤0.05). Pretreatment with TUDCA significantly reduced IL-1β expression compared to the CP-treated group (P≤0.05). (Magnification power = X400, scale bar = 50µm).

DISCUSSION

The liver and kidneys are susceptible to damage from chemotherapeutic agents such as cyclophosphamide, leading to hepatotoxicity and nephrotoxicity (Bhat *et al*., 2018). Antioxidants and anti-inflammatory strategies have shown promise in mitigating these drug-induced organ injuries (Zhai *et al*., 2018).

CP is metabolized into the active metabolites phosphoramide mustard and acrolein by the hepatic microsomal enzymes (Caglayan *et al*., 2018). Among these metabolites, acrolein is cytotoxic and causes the synthesis of nitric oxide and intracellular reactive oxygen species, which in turn causes the production of peroxide (Temel *et al*., 2020). It is well recognized that CP-induced hepatotoxicity and nephrotoxicity are significantly influenced by oxidative stress and elevated reactive oxygen species formation (Caglayanm *et al*., 2018). According to Mahmoud *et al*. (2017), CP-produced free radicals can damage lipids and significantly alter the structure and function of membranes. Furthermore, when acrolein and GSH conjugate, the intracellular GSH level is reduced, which causes oxidative stress (Nafees *et al*., 2018).

The ER has many functions, such as maintaining proteostasis (protein synthesis, folding, modifications, transport, and destruction), lipid synthesis and distribution, carbohydrate synthesis, and calcium storage (Sicari *et al*., 2020). Genetic mutations, transcriptional and translational errors, hypoxia, oxidative stress, hydric and thermal stress, hypoglycemia, heavy metals, chemo-therapeutic agents, or antibiotics are some examples of intrinsic and extrinsic factors that change ER functions and thereby cause ER stress that activates the unfolded protein response (UPR) (Almanza *et al*., 2019).

Chemical chaperones play an important role in the regulation of the UPR, that improves protein folding. TUDCA is one of these chemical chaperones that inhibit ER stress by blocking the activation of the UPR (Gómez-Sierra *et al*., 2021). However, many additional mechanisms, including anti-oxidative, anti-apoptotic, and anti-inflammatory ones, are currently being proposed to explain TUDCA's cytoprotective action (Sankrityayan *et al*., 2023).
According to our findings, TUDCA protects the liver and kidney tissues from CP-induced oxidative stress and lipid peroxidation by reducing free radical damage and oxidative stress and by boosting the antioxidant system, which includes MDA, CAT, and GSH.

This study investigated the hepatotoxic effects of CP in rats, as evidenced by elevated serum levels of liver enzymes (ALT, AST, ALP) and decreased serum albumin and total protein concentrations. These findings align with previous studies indicating CP-induced liver dysfunction and hepatocyte damage (Germoush & Mahmoud, 2014; Patwa *et al*., 2020; Alam *et al*., 2023).

The observed elevations in serum liver enzymes (ALT, AST, ALP) reflect hepatocellular damage and leakage of these enzymes into the bloodstream (Plaa & Hewitt, 2014). Concurrently, decreased serum albumin and total protein levels indicate impaired liver synthetic function (Khalil *et al*., 2020). Previous studies have reported similar findings following CP administration (Yahya *et al*., 2022).

TUDCA treatment effectively ameliorated CP-induced hepatotoxicity by reducing serum liver enzyme levels and increasing serum albumin while decreasing bilirubin (Pan *et al*., 2013; Ma *et al*., 2016).

These findings align with previous in vitro studies demonstrating TUDCA's potent

antioxidant properties (Kusaczuk, 2019; Alabi *et al*., 2021). According to Vandewynckel *et al*. (2015), a diethyl nitrosamine-induced mouse model of hepatocellular cancer showed a substantial decrease in ALT and AST levels when 300 mg/kg/day TUDCA was administered. These were further reinforced by earlier research indicating that TUDCA reduces ER stress to act as a liver-protective agent (Hou *et al*., 2017).

CP induced significant renal dysfunction, as evidenced by elevated serum urea and creatinine levels. These findings align with previous reports linking CP-induced renal injury to increased protein catabolism and impaired renal function (Khalil *et al*., 2020; Alabi *et al*., 2021; Yahya *et al*., 2022; Ali & Mohammed, 2023). TUDCA treatment effectively ameliorated CP-induced renal dysfunction. This renoprotective effect is likely attributed to TUDCA's chaperoning potential, owing to which it reduced the ER stress (Sankrityayan *et al*., 2023). Additionally, CP administration resulted in significant anaemia and leukopenia due to bone marrow suppression and myelotoxicity (Alvi *et al*., 2020; Mirazi *et al*., 2021; Kang *et al*., 2022; Peng *et al*., 2022). The underlying mechanism may involve oxidative stress-induced damage to hematopoietic cells (Khalil *et al*., 2020). TUDCA pretreatment effectively reversed CP-induced haematological alterations, suggesting its potential role in protecting the hematopoietic system. Previous studies reported that TUDCA can increase immature erythroid cell number, which may lead to increased RBCs and HGB levels, as detected in rats treated with TUDCA in our study. The improved WBC level seen here may be due to the ability of TUDCA to preserve cell homeostasis by dramatically lowering lymphocyte apoptosis (Aslan *et al*., 2021).

CP-induced hyperglycemia was successfully mitigated by TUDCA treatment, aligning with previous studies demonstrating TUDCA's glucose-lowering effects (Osama *et al*., 2015; Zhang *et al*., 2016; Khodeer *et al*., 2020; da Silva *et al*., 2021). Furthermore, TUDCA ameliorated CP-induced dyslipidemia, characterized by elevated cholesterol and triglyceride levels and reduced HDL-cholesterol. These findings are consistent with previous research (Rouki *et al*., 2024). The observed increase in lipid peroxidation, as indicated by elevated MDA levels and decreased antioxidant enzyme activities (CAT, GSH), highlights the oxidative stress induced by CP (Germoush & Mahmoud, 2014; Alshahrani *et al*., 2022; Hu *et al.,* 2022; Ali & Mohammed, 2023; Golmohammadi *et al*., 2023). TUDCA's antioxidant properties effectively countered this oxidative stress, as evidenced by reduced MDA levels and increased antioxidant enzyme activities (Weng *et al*., 2024).

Histopathological examination of the liver revealed severe damage, including congestion, necrosis, and loss of tissue architecture. These findings corroborate the observed biochemical alterations and oxidative stress. Similar hepatic changes have been reported following CP exposure (El-Naggar *et al*., 2017; Amiri *et al*., 2018; Elwan *et al*., 2020). The underlying mechanisms likely involve direct cellular toxicity and oxidative stress-induced damage (Khalil *et al*., 2020). Similarly, the kidneys exhibited marked histopathological damage characterized by tubular necrosis, glomerular injury, and interstitial oedema. These findings align with previous reports of CP-induced nephrotoxicity (Osama *et al*., 2015; Alvi *et al*., 2020; Peng *et al*., 2022). The observed renal damage is likely attributed to direct toxic effects on renal tubular epithelium and impaired glomerular filtration (Khalil *et al*., 2020). Importantly, TUDCA treatment significantly ameliorated both hepatic and renal histopathological changes, supporting its protective role against CP-induced organ injury (De Miguel *et al*., 2014).

Cytokines play crucial roles in various physiological processes, including inflammation and immune responses. CP has been shown to induce an inflammatory response in multiple organs (Ayza *et al*., 2022; Turedi *et al*., 2023). Our findings demonstrate increased IL-1β expression in both liver and kidney tissues following CP administration, consistent with previous reports (Turedi, 2023). TUDCA treatment significantly attenuated IL-1β expression in these organs. These results suggest that CP-induced inflammation contributes to tissue damage, and TUDCA's anti-inflammatory properties may underlie its protective effects. The observed reduction in IL-1β expression by TUDCA is in line with its reported ability to prevent cell death (Turedi, 2023). TUDCA suppressed ER stress in Kupffer cells through the IRE1/TRAF2/NF-κB pathway, which reduced the expression and release of pro-inflammatory cytokines IL-1β, IL-6, and TNF. Similarly, in a model of non-alcoholic fatty liver disease, TUDCA enhanced intestinal barrier function by raising levels of tight junction molecules and the solid chemical barrier and reduced gut inflammatory responses by down-regulating pro-inflammatory cytokines such as IL-1β, Ccl2, and Ccl4. This influence was most likely dependent on the reduction of ER stress (Kusaczuk, 2019).

Additionally, TUDCA's antioxidant properties likely contribute to its anti-inflammatory effects by reducing reactive oxygen species (ROS) levels (Alam *et al*., 2023). While this study has some limitations, including the use of an animal model, its findings indicate the potential of TUDCA as a protective agent against CP-induced hepato-renal damage. Although body weight, liver, and kidney weights were not assessed, future research should include a comprehensive evaluation of CP-induced systemic and organ-specific toxicity, along with the protective effects of TUDCA. Additionally, further studies are needed to explore its effects in humans across different age groups to validate these preliminary findings.

Overall, the present study demonstrated that TUDCA protected liver and kidney tissues from CP-induced pathological injury, function impairment, oxidative stress, and inflammatory response, in addition to ER stress. These findings showed the protective effects of TUDCA against CP-induced hepato-renal damage, though further studies are required to validate its relevance in human conditions.

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حمض تورو- أورسو ديوكسيكوليك يخفف من السمية الكبدية الكلوية الناتجة عن السيكلوفوسفاميد في الفئران: تخفيف الاجهاد التأكسدي والالتهاب

أميرة سمير ، أشرف الكومي ، ايناس فراج ، أحمد عابدين

Email: amirasamir01@yahoo.com; ahmed.abdeen@fvtm.bu.edu.eg
Assiut University web-site: www.aun.edu.eg

يعد عقار السيكلوفوسفاميد من الادوية الكيميائية العلاجية القوية لعلاج العديد من الأورام الخبيثة الصلبة والدموية ويسبب سمية كبدية وكلوية وقلبية وغيرها، مما يحد من استخدامه. المكمل الغذائي حمض تورو- أورسو ديوكسيكوليك هو احد مضادات الاكسدة و مضاد ايضا للالتهابات. كان الغرض من هذه الدراسة هو التحقق من صحة التأثير الوقائي لحمض تورو- أورسو ديوكسيكوليك على السمية الكبدية والكلوية الناجمة عن السيكلوفوسفاميد . تم استخدام أربعة وعشرين (٢٤) ذكرًا من الجرزان البيضاء (١٦٠ - ٢٠٠ جم) لهذه التجربة. قد تم تقسيمهم عشوائيًا إلى أربع مجموعات .المجموعه الأولى (الضابطة): تم اعطاء الجرذان محلول ملح عن طريق الفم مرة واحدة يومياً لمدة اربعة عشر يوماً متتاليا. المجموعة الثانية: تم اعطاء الجرذان حمض تورو- أورسو ديوكسيكوليك (٣٠٠ ملليجرام /كجم وزن) عن طريق الفم مرة واحدة يوميا لمدة أربعة عشر يوماً متتاليا. المجموعة الثالثة: تم اعطاء الجرذان محلول ملح عن طريق الفم مرة واحدة يوميا لمدة أربعة عشر يوماً متتاليا وتم حقنها بالسيكلوفوسفاميد (٢٠٠ ملليجرام /كجم وزن) عن طريق البروتون مرة واحدة في اليوم العاشر من التجربة . المجموعة الرابعة: تم اعطاء الجرذان حمض تورو- أورسو ديوكسيكوليك (٣٠٠ ملليجرام /كجم وزن) عن طريق الفم مرة واحدة يوميا لمدة أربعة عشر يوماً متتاليا وتم حقنها بالسيكلوفوسفاميد (٢٠٠ ملليجرام /كجم وزن) عن طريق البروتون مرة واحدة في اليوم العاشر من التجربة.

كشفت هذه الدراسة أن إعطاء السيكلوفوسفاميد إلى تلف كبدي و كلوي حيث اتضح من الزيادة الكبيرة في أنشطة انزيم الآنين امينو ترانسفيريز واسبرتات امينو ترانسفيريز وانزيم الفوسفاتيز القلوي والكرياتينين واليوريا والبيليروبين الكلي والجلوكوز والكوليستيرول والدهون الثلاثية بينما انخفض مستوى البروتين الدهني العالي الكثافة والألبومين والبروتين الكلي وكرات الدم الحمراء والبيضاء والهيموجلوبين والصفائح الدموية في الدم مقارنة بتلك الموجودة في الجرذان الضابطة. قلل العلاج بحمض تورو_اورسو ديوكسيكوليك بشكل كبير من مستويات انزيم الآلنين امينو ترانسفيريز واسبرتات امينو ترانسفيريز وانزيم الفوسفاتيز القلوي والكرياتينين واليوريا والبيليروبين الكلي والجلوكوز والكوليستيرول والدهون الثلاثية في الدم وايضاً ارتفع مستوي البروتين الدهني العالي الكثافة والالبيومين البروتين الكلي وكرات الدم الحمراء والهيموجلوبين والصفائح الدموية في الدم مقارنة بالجرزان المعالجه بالسيكلوفوسفاميد. تعرضت الأنسجة الكبدية والكلوية لأضرار مؤكسدة بسبب العلاج بالسيكلوفوسفاميد ، كما اتضح من الزيادة الكبيرة في مستويات المالونالديهيد MDA وانخفاض نشاط مستويات الكاتاليز CAT والجلوتاثيون GSH وفي الوقت نفسه، كشفت المعالجة المسبقة بحمض تورو_اورسو ديوكسيكوليك أدى إلي انخفاض في مستوى المالونالدهيد MDA جنبا إلى جنب مع الارتفاعات في نشاط مستويات الكاتاليز CAT والجلوتاثيون GSH في الأنسجة الكبدية والكلوية مقارنة بالمجموعة المعالجة بالسيكلوفوسفاميد. وقد أظهر فحص أنسجة كبد الجرذان في المجموعات الضابطة والمجموعات المعالجة بحمض تورو_اورسو ديوكسيكوليك بنية طبيعية لأنسجة الكبد حيث تم ترتيب الخلايا الكبديه ذات النوي المركزية في شكل خطوط تشع من الأوردة المركزية .أظهر الكبد الذي تم فحصه للجرذان المعالجه بالسيكلوفوسفاميد احتقانا كبديا حيث يوجد مناطق تليف متعددة البؤر وتغيركبير في شكل خلايا الكبد التي اظهرت سيتوبلازما شفافاً ونوى مضمحلاً .كما كشف فحص الكبد من الجرذان المعالجة مسبقا بحمض تورو_اورسو ديوكسيكوليك + سيكلوفوسفاميد عن وجود بؤر قليلة متليفة لخلايا الكبد مع احتقان خفيف في الأوردة المركزية .أظهر الفحص المجهري لكلى الجرذان الضابطة و الجرذان المعالجة بحمض تورو_اورسو ديوكسيكوليك بنية نسيجية طبيعية للكبيبات والأنابيب الكلوية مع تغيرات غير ملحوظة .في المقابل، كشفت الكلى التي تم فحصها من الجرذان

المحقونة بالسيكلوفوسفاميد عن ضمور في الانسجة المبطنة للأنابيب الكلوية، ووجود ارتشاح في الخلايا الليمفاوية .وفي الوقت نفسه، تم ظهور تحسن جزئي في أنسجة كلي الجرذان المعالجة سابقا بحمض تورو_اورسو ديوكسيكوليك قبل حقن السيكلوفوسفاميد حيث تم تسجيل القليل من البؤر الضامرة في انابيب الكلي مع وجود تجمعات في تجويف بعض الأنابيب الكلوية .بالإضافة إلى ذلك في التعبير عن بروتين الإنترلوكين ون بيتا (IL-1β) في خلايا الكبد والكلى أكدت المجموعات الضابطة والمعالجة بحمض تورو_اورسو ديوكسيكوليك على التفاعل السلبي مع الأجسام المضادة. على العكس في المجموعة المعالجة بالسيكلوفوسفاميد اظهرت تفاعل قوي مع الإنترلوكين 1β. بينما المجموعات المعالجة سابقا بحمض تورو_اورسو ديوكسيكوليك قبل حقن السيكلوفوسفاميد اظهرت تفاعل معتدل. من هذه الدراسة، يمكن الاستنتاج أن اعطاء حمض تورو_اورسو ديوكسيكوليك كعلاج مساعد مع السيكلوفوسفاميد ساهم في تقليل السمية التي يسببها السيكلوفوسفاميد على الكبد و الكلى بفضل خصائصه المضادة للأكسدة والمضادة للالتهابات.

الكلمات المفتاحية: السيكلوفوسفاميد , ضغوط الاكسدة, تسمم الكبد والكلي, حمض تورو_اورسو ديوكسيكوليك