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Research Article

Pentoxifylline ameliorates carbamazepine-induced hepatotoxicity via down expression of *CYP3A4* and *NF-kB* gene expression in the rat: *in vivo* study

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Abstract

Drug-induced liver injury (DILI) is a serious complication of many drugs, including carbamazepine; this study investigates the protective effect of pentoxifylline (PTX) against DILI induced by carbamazepine in rat models by attenuating *CYP3A4* and *NF-κB* gene expression. Forty rats were divided into five groups: the control group received no treatment, the induction group received 50 mg/kg carbamazepine orally for 28 days, and three groups received PTX (100, 200, and 300 mg/kg) once daily for one hour before carbamazepine induction for 28 days. Then, the rats were euthanized, and blood and liver tissue were collected for biochemical, gene expression, and histopathology. PTX attenuates the carbamazepine-induced increased *CYP3A4* and *NF-κB* gene expression, with the highest dose showing the best result. PTX also reduced aspartate aminotransferase, alanine aminotransferase, and malondialdehyde, while glutathione levels increased. In conclusion, PTX is highly efficacious in preventing and restoring the liver cells' normal morphology and cellular function caused by carbamazepine hepatotoxicity. A possible mechanism of the PTC effect is hindering oxidative stress by scavenging free radicals and enhancing the body's natural defense antioxidant system. Additionally, it exerts an anti-inflammatory impact by modulating *NF-κB* gene expression.

Keywords

carbamazepine, CYP3A4, gene expression, hepatotoxicity, NF-κB, pentoxifylline

Introduction

Adverse drug reactions (ADR) are a significant factor in causing liver damage, which may necessitate stopping the harmful medication, hospitalization, or, in severe cases, liver transplantation (Smith and Schmid 2006; Almazroo et al. 2017). Drug-induced liver injury (DILI) is the leading cause of acute liver failure (Ostapowicz et al. 2002). The function of the liver as the primary detoxifying organ for many drugs incidentally makes it the main target for these drugs to cause liver damage (David and Hamilton 2010; Katarey and Verma 2016; Alizzi et al. 2018; Saad et al. 2022).

DILI is commonly categorized as either intrinsic or idiosyncratic (Hosack et al. 2023). Intrinsic DILI usually occurs in a dose-dependent manner and follows a predictable pattern, with hepatotoxicity appearing within hours

to days after exposure (EASL 2019). Drugs that cause intrinsic DILI are frequently lipophilic, allowing them to pass through the lipid bilayer of hepatocytes easily. They experience biomodification, transforming into reactive compounds that trigger oxidative stress and stimulate cellular signaling pathways, leading to mitochondrial dysfunction and disruptions in bile acid balance (EASL 2019). In contrast, idiosyncratic DILI usually has an unexpected progression. It can occur anytime from a few weeks to months after exposure to the drug (Chalasani et al. 2014), which may be associated with the adaptive immune system and specific human leukocyte antigen (Dara et al. 2016). DILI is a serious clinical consequence that arises during the treatment and diagnosis of several pathological conditions (Intesar Tarik et al. 2022). Several drugs are associated with DILI, such as antidepressants (González-Muñoz et al. 2020), teriflunomide (Wurzburger 2022), tigecycline (Shi et al. 2022), amiodarone (Fadhil et al. 2024), and potassium para-aminobenzoate (Plüß et al. 2022).

Oxidative stress (OS) occurs when reactive oxygen species (ROS) are produced as a by-product of regular metabolism. These ROS play important functions in cellular signaling and maintaining internal balance. Certain medicines that cause DILI can enhance the formation of ROS in multiple ways (Ye et al. 2018). Furthermore, in the development of DILI, the liver experiences an increase in mitochondrial H_2O_2 due to the depletion of GSH (glutathione) (Ye et al. 2018). Lipid peroxidation (LPO) may play a role in the cell death process in DILI. During LPO, the formation of lipid radicals results in the degradation of polyunsaturated fatty acids (PUFAs) in lipid membranes (Walgren et al. 2005). LPO can result in the abrupt and severe disruption of the membrane potential and ion gradients, ultimately resulting in necrotic cell death (Wendel et al. 1979). The occurrence of cell damage caused by LPO necessitates both the generation of oxidants and the dysfunction of antioxidant defense mechanisms (Jaeschke and Ramachandran 2018).

The clinical manifestation of DILI exhibits a wide range of severity and phenotypic expression, spanning from an asymptomatic increase of liver enzymes to the development of acute liver failure (ALF). Liver biopsy is typically not utilized to diagnose DILI. Instead, liver biochemistry is employed to determine the specific type of liver damage. DILI is usually defined as an increase in serum alanine aminotransferase (ALT) levels that are at least 5 times higher than the upper limit of normal (ULN), an increase in serum alkaline phosphatase (ALP) levels that are at least 2 times higher than the ULN, or a combination of ALT levels that are at least 3 times higher than the ULN along with a contemporary increase in total bilirubin (TBL) levels that are more than 2 times higher than the ULN (Benichou and Solal Celigny 1991; Andrade et al. 2019; EASL 2019).

Epileptic seizures are one of the most common chronic neurological disorders. Treated with antiepileptic medicines, like carbamazepine (CBZ); however, CBZ is usually associated with increased liver enzymes (Osuntokun et al. 2021b). CBZ is ionized in the intracellular fluid and shares structural similarities with tricyclic antidepressants. The most significant pathway for CBZ metabolism is the active metabolite CBZ-10,11-epoxide, produced by cytochrome P450 (CYP) isoenzymes, especially CYP3A4 and 3A5. This metabolite is assumed to contribute to the toxicity of CBZ (Kacirova et al. 2021).

CYP3A4 is crucial for the metabolism of CBZ and the production of CBZ 10–11 epoxide, the active CBZ metabolite. Epoxide hydrolase 1 (EPHX1), which encodes the microsomal epoxide hydrolase, is another significant CBZ metabolizer. The primary metabolizer of CBZ, EPHX1, converts CBZ 10,11-epoxide into the inactive, water-soluble metabolite CBZ 10,11-diol (Iannaccone et al. 2021). Other metabolites include 3-hydroxycarbamazepine (3OH-CBZ) and 2-hydroxycarbamazepine (2OH-CBZ). Myeloperoxidase (MPO) facilitates the secondary oxidation of CBZ-3-OH, which produces CBZ-3-OH free radicals. Oxidation of CBZ-2-OH leads to the formation of 2-OH iminostilbene. Additionally, it is believed that the conversion of CBZ to arene oxide plays a significant role in the hepatotoxicity caused by this drug (Jaramillo et al. 2014). The synthesis of these metabolites involves several CYP isoforms (Yip et al. 2021). Moreover, inducers and inhibitors of some CYP enzymes can alter the plasma levels of CBZ. Thus, complex interaction patterns may arise when CBZ is co-administered with other medications (Fuhr et al. 2021).

Pentoxifylline (PTX) is a methylxanthine medication that has been utilized in clinical practice for medical purposes such as peripheral arterial disorders(PAD) (Broderick et al. 2020). It also improves blood flow by opening blocked capillaries and microcirculation (Khairy et al. 2023). The anti-inflammatory property of PTX has been used to treat liver disorders by blocking phosphodiesterase-4 (PDE-4), increasing the intracellular concentration of cyclic adenosine monophosphate (cAMP); it also possesses an antioxidant property (Ahmed et al. 2012; Li et al. 2018). PTX was shown to protect the liver from chemically induced liver damage (Luo et al. 2015); some suggested this protection is associated with proinflammatory and anti-inflammatory cytokines, both strongly inhibited by PTX (Osuntokun et al. 2021b); however, the exact mechanism is still unknown.

This is the first study to examine the protective effects of PTX in DILI induced by CBZ, proposing that this effect is mediated via its activity on CYP3A4 and NF-κB gene expression in rat animal models. In addition to studying gene expression, the biochemical parameters for liver function enzymes, oxidative stress markers, and histopathological findings were examined to elucidate the molecular mechanisms for PTX activity.

Methods

Ethical approval

The Scientific Committee of Mustansiriyah University's College of Pharmacy, Pharmacology, and Toxicology Department granted ethical approval for this study (approval number: 24, reference number: 102, date: 28 October 2023). Animal handling, experimental procedures, and euthana-

sia were carried out according to the guidelines for animal care of the National Institute of Health and the American Veterinarian Medical Association (AVMA) 2020 (Underwood and Anthony 2020). The authors complied with the ARRIVE 2.0 guidelines (Percie du Sert et al. 2020).

Study design and settings

This investigation, aimed at studying the effects of specific substances on male rats, carefully selected forty rats 8–11 weeks of age, weighing between 130 and 150 g. The rats were kept in large, comfortable cages, brought from the Iraqi Center for Cancer Research, and placed in the animal house at Mustansiriyah University, College of Pharmacy. They spent seven days in a regulated setting with an average temperature of 25 ± 1 °C, 40–50% humidity, and a 12-hour light/dark cycle. The rats had unrestricted access to food and water. The study was conducted at the animal house in the College of Pharmacy, Mustansiriyah University, between 28 October 2023 and 24 November 2023.

Five groups of eight rats per each were formed randomly using a complete block design: group 1 (normal control) received distilled water (DW) 10 mL/kg daily orally for 28 days (Bouhrim et al. 2021), group 2 (induction) received 50 mg/kg CBZ orally via gastric gavage for 28 days (Maheswari et al. 2014; Osuntokun et al. 2021a), group 3 (PTX100): received 100 mg/kg PTX orally via gastric gavage one hour before CBZ induction for 28 days (Kor et al. 2018), group 4 (PTX200): received 200 mg/kg PTX orally via gastric gavage one hour before CBZ induction for 28 days (Mohamed et al. 2014), and group 5 (PTX300): received 300 mg/kg PTX orally via gastric gavage one hour before CBZ induction for 28 days (Khairy et al. 2023), as illustrated in Fig. 1.

Pentoxifylline powder (Meryer, China) is freely soluble in saline; it was used as a freshly prepared aqueous solution (Hendawy 2017). Carbamazepine powder (Meryer, China) was dissolved in corn oil (Sasaki et al. 2016).

After the experimental phase of the study, all the animals were euthanized using anesthesia with a combination of ketamine and xylazine. Specifically, the rats were anesthetized intraperitoneally with 80 mg/kg of ketamine (Ketamine 10%, Alfasan Nederland BV, Holand) and 10 mg/kg of xylazine (XYL-M2, VMD® Livestock Pharma, Belgium). Once the animals were fully anesthetized, they passed away by exsanguination through cardiac puncture. This method was chosen as it allows for effective tissue harvest and preservation (Obaid and Fawzi 2024). Blood and tissue samples were collected for further analysis.

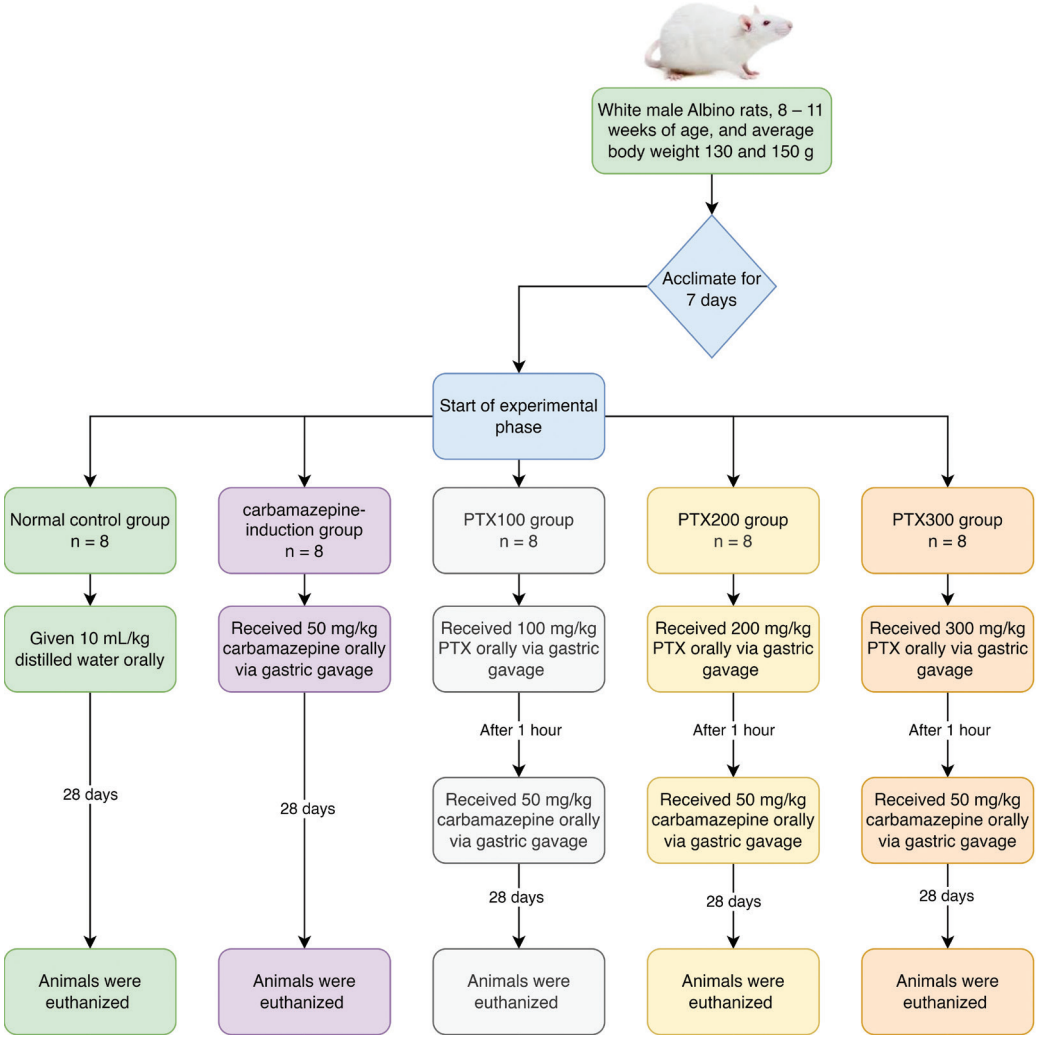

Figure 1. Flow chart of the study.

Special considerations to minimize the suffering and distress of animals

The animals in the study received treatment for any external injuries involving their skin using appropriate skin creams to prevent potential secondary infections. Additionally, a low dose of xylazine (3 mg/kg, IP) was administered when the animals exhibited signs of stress (Khafaji et al. 2024).

Sample collection

Blood samples were collected from the animals through heart puncture (Diehl et al. 2001; Hussein et al. 2024b). After blood was collected, the sample was spun at a speed of 5,000 revolutions per minute for 10 minutes to separate the serum. The serum was collected in 2 ml Eppendorf tubes and kept at a temperature of -20 until the analysis day. Following the animals' sacrifice, a liver tissue sample was obtained. The tissues were then stored in a neutral buffered formalin solution (10%) and underwent histological inspection.

Assessment of biochemical analysis

The activities of colorimetric test kits measuring aspartate aminotransferase (AST) and ALT in serum samples were done using commercially available diagnostic kits (Linear Chemical, Spain). The enzyme-linked immunosorbent assay (ELISA) approach was used to measure the oxidative stress markers in the liver. As a result, malondialdehyde (MDA) (Linear Chemical, Spain), the end-product of lipid peroxidation, was used to quantify reactive species. Also, the antioxidant marker (GSH) (Linear Chemical, Spain) was measured using the ELISA method (Salahshoor et al. 2020).

Determination of mRNA expression of CYP3A4 and NF-KB in liver tissue

The real-time quantitative polymerase chain reaction (RT-PCR) method, which involves liver tissue isolation, total RNA extraction and purification, cDNA production, and RT-PCR procedures, was used to determine the gene expression levels of *CYP3A4* and *NF-κB* in liver tissue. Data analysis was then conducted. β-actin was used as a housekeeping gene. Following a 28-day treatment with CBZ, the rats' livers were removed, and TRIzol® was used to extract the RNA. After that, the RNA was reverse-transcribed to cDNA after being diluted with RNase-free water (Bai et al. 2021).

A mechanical homogenizer was used to homogenize the tissue, and centrifugation was performed. Then, the supernatant was mixed with 200 µL of chloroform and vigorously shaken for 30 seconds. Then, incubate for three minutes. Following a 15-minute centrifugation at 10,000 rpm and 4 °C, the mixture was separated into three layers: an upper containing the RNA. After pipetting and transferring into a fresh tube devoid of RNase, 600 µL of ethanol was added (to facilitate RNA isolation). The mixture was run through a spin column and centrifuged for one minute at 4 °C at 12,000 rpm; 500 µL of clean buffer (CB9) was added and centrifuged for one minute at 12,000 rpm to remove impurities from the RNA (this step was repeated twice). Subsequently, 500 µL of wash buffer (WB9) was added to remove contaminants, rotating twice at 12,000 rpm for one minute at 4 °C. Then, centrifuged for two minutes at 12,000 rpm, the column matrix was allowed to air dry. Then, 120 µL of RNase-free water was added (Hussein et al. 2024a).

Table 1 lists the primers created and the RNA primer mix preparation by adding the following(RNA template, forward primer, and reverse primer) and testing for annealing temperatures for this investigation.

Histopathological examination

The liver tissues were fixed in 10% neutral formalin overnight. The tissues were dehydrated in ethanol, cleared in xylol, and then embedded in paraffin blocks using standard procedures. Three µm-thick paraffin slices of the liver were cut using a microtome. Sections were stained with hematoxylin and eosin (H&E) (Hamouda et al. 2022).

Two independent pathologists who were unaware of the study groups examined the grading of the disease activity; numerical values were assigned to this semiquantitative score, as illustrated in Table 2 (Batts and Ludwig 1995).

Table 1. The primer sequence of genes used in this study and suppliers.

Gene	Forward primer sequence(5′→3′direction)	Reverse primer sequence(5′→3′direction)	Supplier
β-actin	CTATCGGCAATGAGCGGTTCC	TGTGTTGGCATAGAGGTCTTTACG	Alpha-DNA, Canada
NF–κB	GTCATCAGGAAGAGGTTTGGCT	TGATAAGCTTAGCCCTTGCAGC	Alpha-DNA, Canada
CYP3A4	TCTGTGCAGAAGCATCGAGTG	TGGGAGGTGCCTTATTGGG	Alpha-DNA, Canada

Table 2. Grading of disease activity. [#]

Grading terminology		Criteria	
Semiquantitative	**Description**	**Lymphocytic piecemeal necrosis**	**Lobular inflammation and necrosis**
0	Portal: inflammation only; no activity	None	None
1	Minimal	Patchy	Occasional spotty necrosis
2	Mild	Involving some or all portal tracts	Little hepatocellular damage
3	Moderate	Involving all portal tracts	With noticeable hepatocellular damage
4	Severe	It may have bridging fibrosis	With prominent diffuse hepatocellular damage

[#] The more severe lesion should be used to determine the grade when there is a discrepancy between both criteria.

Sample size calculation and animal randomization

The software program G.Power was employed to calculate the sample size (Faul et al. 2007; Charan and Kantharia 2013). Random numbers were employed to generate groups in an Excel spreadsheet. The rats were placed in labeled containers and assigned tail tags to minimize confusion (Festing 2006).

Statistical analysis

Descriptive and inferential statistics were performed using GraphPad Prism 10.3; the normality test (Anderson Darling test) was performed initially to determine the continuous variable's adherence to a normal distribution. The parametric variable was analyzed using the ANOVA and post hoc Tukey tests. In contrast, the non-parametric variable was analyzed using the Kruskal-Wallis and post hoc Dunn tests. 0.05 was considered the level of significance for the current study.

Results

Effect of PTX on serum AST and ALT activity

Serum AST and ALT levels were significantly higher in the induction groups than in the control group and significantly lower in the treatment groups than in the induction group. PTX at 300 mg/kg dose showed the lowest AST and ALT levels compared to the other treatment groups, as illustrated in Fig. 2A, B.

Effect of PTX on Serum MDA and GSH

Serum MDA level was significantly higher in the induction groups than in the control group and significantly lower in the treatment groups than in the induction group. PTX at a 300 mg/kg dose showed the lowest MDA level compared to the other treatment groups, as illustrated in Fig. 2C.

Serum GSH was significantly lower in the induction group than in the control group; PTX at a dose of 200 and 300 mg/kg showed significantly higher GSH than the induction group, as illustrated in Fig. 2D.

Effect of PTX on CYP3A4 and NF-KB gene expression in liver toxicity

CBZ treatment increases the expression of *CYP3A4* and *NF-κB* gene expression compared to the control group; PTX at a dose of 300 mg/kg significantly reduces the gene expression of *CYP3A4* compared to the induction group, as illustrated in Fig. 3A. PTX at doses of 100, 200, and 300 mg/kg significantly reduces the gene expression of *NF-κB* compared to the induction group, as illustrated in Fig. 3B.

Histological changes

A liver histopathology examination was conducted to confirm the biochemical changes in the liver, as shown in Fig. 4. In the control group, the liver section shows a

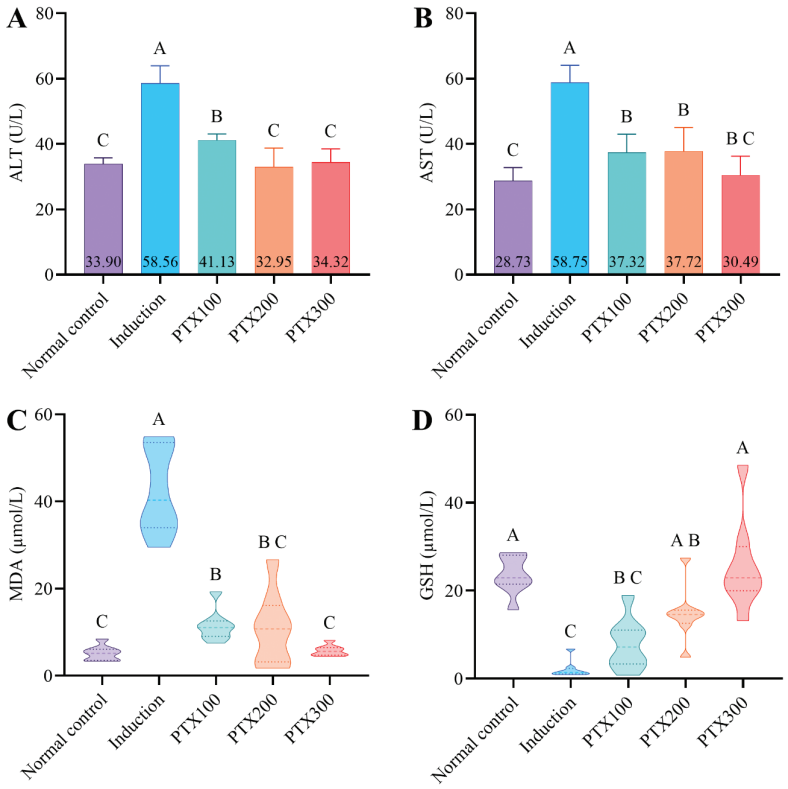

Figure 2. Assessment of serum levels of biomarkers according to the study groups. **A** ALT **B** AST **C** MDA **D** GSH.

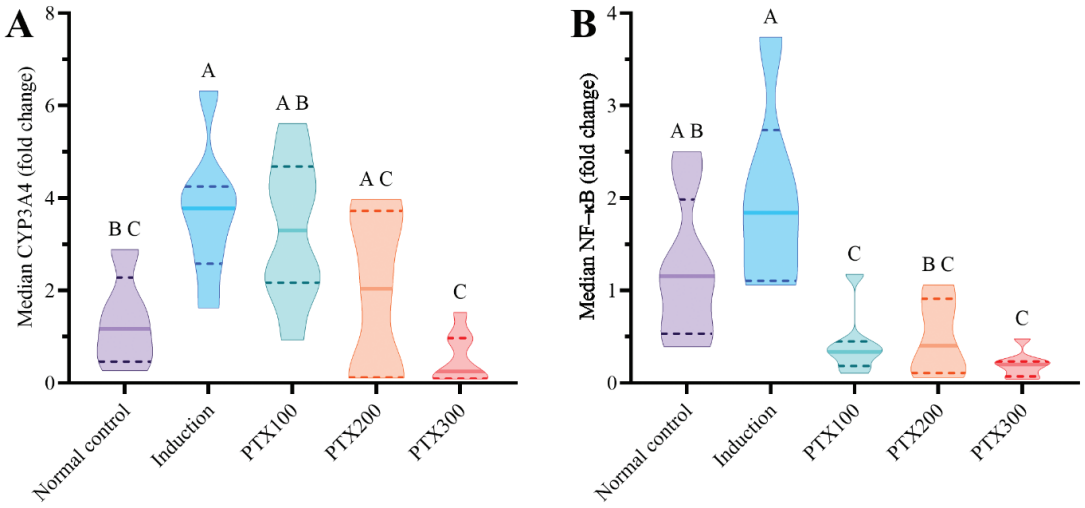

Figure 3. Violin plot of the gene expression according to treatment groups. **A** *CYP3A4* gene expression **B** *NF-κB* gene expression.

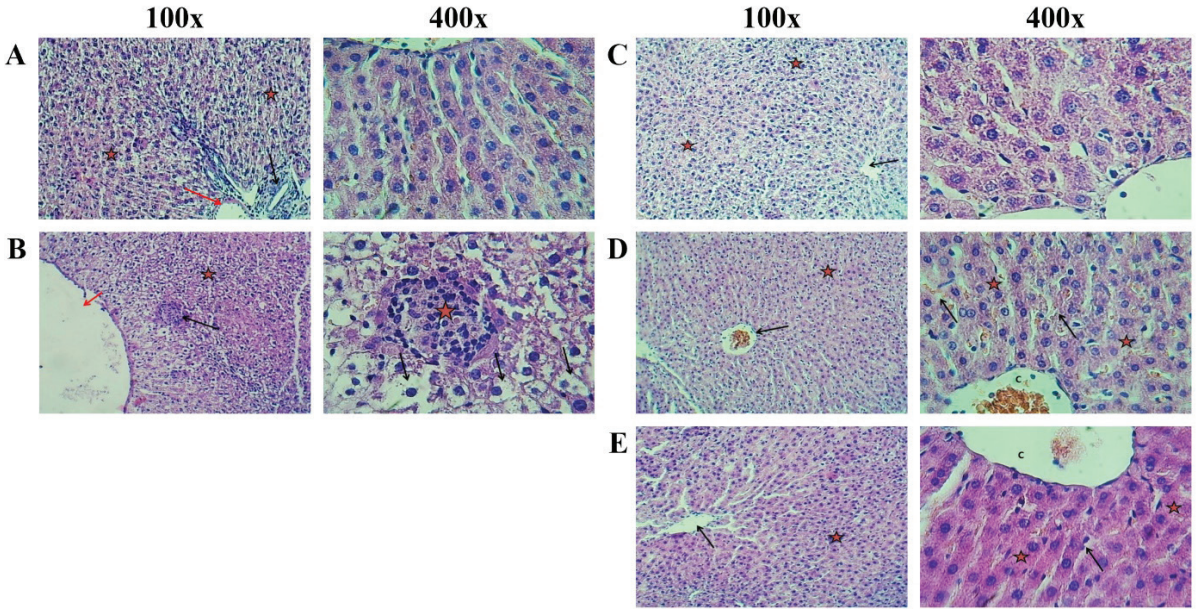

Figure 4. Histopathological section of the liver. Liver slices were stained with hematoxylin and eosin for histological investigation of the effects of CBZ and PTX on the liver. **A** Control group **B** induction group **C** the PTX100 group **D** the PTX200 group **E** the PTX300 group. H&E stain at 100× and 400× power.

typical appearance of the portal vein, standard bile duct, and normal appearance of hepatocytes (Fig. 4A). The induction group shows massive dilation of the central vein, generalized swelling of hepatocytes, and several areas of multiple focal necrosis cell death and accumulation of mononuclear cells (Fig. 4B). PTX100 group shows a usual central vein, as indicated by an arrow. Additionally, hepatocytes have a slight zonal cellular enlargement without necrosis (Fig. 4C). The PTX200 group displays a usual central vein with minimal congestion and normal hepatocytes with slight sinusoidal congestion (Fig. 4D). PTX300 group, this portion of the liver displays the following features: the central vein appears normal, the hepatocytes also appear normal, and the sinusoids are indicated by an arrow (Fig. 4E).

Comparison of histological scores among study groups

A comparison of histological scores among study groups is shown in Table 3. In most of the scores (portal area, lobar area, degenerative & necrosis, venous & sinusoidal congestion, proliferation of cholangiocytes, and infiltration of MNC (mononuclear cells), the induction group showed the highest median score compared to the other groups.

PTX showed a dose-dependent reduction in the inflammatory scores in the portal and lobar areas, degenerative and necrosis, venous and sinusoidal congestion, proliferation of cholangiocytes, and infiltration of MNC, with the higher doses showing better reduction (in descending order 300, 200, and 100 mg).

Table 3. Assessment of histological scores.

Characteristic	Control group	Induction group	PTX100	PTX200	PTX300	*p-value*
Portal area						
Median (IQR)	0 (0) ^{B}	4 (1) ^{A}	0 (1) ^{B}	0 (0) ^{B}	0 (1) ^{B}	0.001
Range	0–0	3–4	0–1	0–0	0–1	
Lobar area						
Median (IQR)	0 (0) ^{B}	3 (1) ^{A}	0 (1) ^{B}	0 (0) ^{B}	0 (1) ^{B}	0.001
Range	0–0	3–4	0–1	0–0	0–1	
Degeneration & Necrosis						
Median (IQR)	0 (0) ^{C}	4 (1) ^{A}	3 (1) ^{B}	0 (0) ^{C}	0 (1) ^{C}	<0.001
Range	0–0	3–4	2–3	0–0	0–1	
Venous & sinusoidal congestion						
Median (IQR)	0 (1) ^{C}	3 (1) ^{A}	2 (0) ^{B}	1 (0) ^{B}	1 (1) ^{B}	0.001
Range	0–1	2–3	1–2	1–2	1–1	
Portal or lobular Fibrosis						
Median (IQR)	0 (0) ^{B}	0 (0) ^{B}	1 (1) ^{A}	0 (0) ^{B}	0 (0) ^{B}	0.011
Range	0–0	0–0	0–1	0–0	0–0	
Proliferation of Cholangiocytes						
Median (IQR)	0 (0) ^{D}	4 (0) ^{A}	2 (2) ^{B}	0 (0) ^{D}	1 (2) ^{C}	<0.001
Range	0–0	3–4	1–3	0–1	0–2	
Infiltration of MNCs						
Median (IQR)	0 (0) ^{B}	3 (3) ^{A}	0 (0) ^{B}	0 (1) ^{B}	0 (1) ^{B}	0.001
Range	0–0	3–3	0–0	0–1	0–0	

Kruskal-Wallis test was used, and Dunn's post hoc was used for multiple comparison.
The significance level was indicated with capital letters. There is no significant difference when two letters are similar; when they differ, there is. The letter A represents the greatest mean value, followed by B, C, D, and E.
IQR: inter-quartile range, MNCs: mononuclear cells

Discussion

When selecting medications to treat epileptic seizures, the liver is still one of the essential organs that must be given extra thought. CBZ, first-line AEDs produce toxicity and raise liver enzymes (Osuntokun et al. 2021b). The liver's integrity was assessed by measuring the liver enzymes, ALT and AST; the level of these enzymes in the group receiving CBZ in the current investigation was higher than in the other groups. Hepatic damage results in the leakage of liver enzymes into the bloodstream and raises their level in serum, indicating liver dysfunction (Salahshoor et al. 2020). These findings are consistent with other research (Kareem et al. 2022). All treatment groups showed significantly lower AST and ALT levels than the induction groups, indicating that PTX significantly dropped ALT and AST serum activity; this agrees with a previous study demonstrating that PTX treatment reduced hepatic damage (Ali et al. 2018).

This investigation showed that CBZ use increased the OS and decreased antioxidant enzyme activity. An imbalance between the oxidative and antioxidant systems was caused by long-term use of CBZ, which led to a significant increase in OS markers such as MDA and a reduction in GSH. In the present study, serum MDA levels were significantly higher in the induction group than in the control group, which is associated with the generation of ROS. A previous study showed that an excess of ROS causes cell death by apoptosis, which explains why apoptosis is higher after exposure to CBZ (Yan et al. 2021). In the present study, the MDA level decreased significantly in the PTX treatment groups compared to the induction group; this agrees with another study showing no enhancement in MDA plasma levels following PTX treatment in patients who experienced a recent cardiac event (Saeed et al. 2024).

Disruption of physiologically active proteins, DNA, or phospholipids produces free radicals, which generate hydroperoxides and endoperoxides. These compounds can further decompose to form reactive intermediates such as MDA, causing cell death or the inactivation of vital cellular processes (El-Mowafy et al. 2022).

The results of the present study show a decrease in GSH levels in the CBZ group compared to the control group, which is consistent with another study (Kareem et al. 2022); extended overproduction of free radicals and OS may be compensated by increased GSH activity (Khairy et al. 2023).

Treatment with PTX significantly increased GSH levels in the current study, supporting cell survival and preserving the body's natural antioxidant defenses. The potential of PTX to upregulate the Nrf-2 transcription factor may account for its more substantial influence on GSH (Ali et al. 2018). Additionally, because of its actions in scavenging free radicals, PTX decreased MDA, contrary to the findings of another study, which revealed a rise in MDA levels and no enhancement in the reduction of GSH in ischemia/reperfusion (Demir and Inal-Erden 1998). ROS can alter signaling effectors, including enzymes and transcription factors like NF-κB, specifically affecting cell division, proliferation, and apoptosis, ultimately resulting in cell disruptions (El-Mowafy et al. 2022).

NF-kB is activated during or immediately before cell apoptosis (programmed cell death) under stimulatory conditions, suggesting that the activation of NF-κB is

strongly linked to the inhibition of apoptosis (Serasanambati and Chilakapati 2016). The prototypical activators of the NF-κB pathway are comprised of tumor necrosis factor α (TNF-α). NF-κB can interfere with apoptotic signals at various levels. The first pathway in which the NF-κB interferes with apoptosis is by inducing transcription of a set of genes coding for anti-apoptotic proteins (c-FLIP), which is under NF-κB control; upon induction, c-FLIP associates with the TNF receptor to compete with and block caspase-8 activation and thereby inhibit apoptosis. The second pathway by which NF-κB interferes with the apoptotic pathway in the mitochondria is that NF-κB transcribes the genes coding for several anti-apoptotic proteins that act to prevent cytochrome-c release and the subsequent caspase-9 activation, thereby inhibiting apoptosis (Serasanambati and Chilakapati 2016).

PTX can reduce the morbidity and mortality brought on by liver toxicity by its anti-inflammatory properties (Ali et al. 2018). PTX down-regulates NF-κB activity by diminishing phosphorylation and degradation of inhibitory complex I-κBα, nuclear translocation, and DNA binding of NF-κB. It possesses strong anti-inflammatory effects as it downregulates TLR4 signaling in monocytes (Khan et al. 2022).

In the current investigation, NF-κB was significantly upregulated after 28 days of CBZ administration. While the administration of PTX significantly reduced the up-regulation of NF-κB, these findings align with another study (Ali et al. 2018). The protective effects of PTX against CBZ-DILI could be attributed at least partly to the anti-inflammatory effects that appear in their ability to inhibit NF-κB.

The treated group (100 mg/kg) showed a slight decline in *CYP3A4* level, the same as the PTX-treated group (200 mg/kg). Still, the major decline significantly occurs with the PTX-treated group (300 mg/kg), which decreases the biotransformation of CBZ and damages the hepatocytes. *CYP3A4* and *CYP3A5*, two human *CYP3A* enzymes, are considered the most significant for drug metabolism; both are highly prevalent in the liver and gut (Saiz-Rodríguez et al. 2020). CYP3A4 is implicated in the biotransformation of multiple medications and continues to be a key player in the metabolism of numerous drugs, such as CBZ. CBZ substantially induced *CYP3A4* (Kang et al. 2008). In the present study, *CYP3A4* in the induction group was significantly higher than in the PTX groups, particularly at 300 mg/kg. PTX shows a decrease in *CYP3A4* gene expression level.These results disagree with another study that shows the induction of *CYP3A* reached a higher level after 14 days of CBZ administration, then significantly decreased after 28 days of CBZ (Yamashita et al. 2002).

Histological changes encompass cellular apoptosis and the development of fibrous formations, resulting in the contraction and concealment of hepatic cells. The hepatocytes of rats' livers subjected to CBZ poisoning exhibited significant damage and necrotic changes, consistent with the investigation results (Ali et al. 2022). The animals treated with CBZ showed a variety of degenerative alterations in their hepatic parenchyma, as observed in histological findings. At the same time, PTX groups revealed a significant improvement in the liver. Furthermore, higher doses of PTX had a more significant impact on restoring the standard shape of hepatocytes. The results align with the findings (Hamouda et al. 2022).

Study limitations

Although animal models offer significant insights into human illnesses, they may not completely represent the intricate nature of male infertility in humans. The research did not employ clinical data obtained from human participants. Despite the encouraging results in the rat model, more investigation is required to ascertain the safety and effectiveness of pentoxifylline in humans.

Conclusions

Overall, the findings indicate that PTX is highly efficacious in preventing and restoring the liver cells' normal morphology and cellular function caused by carbamazepine hepatotoxicity. A possible mechanism of the PTC effect is hindering oxidative stress by scavenging free radicals and enhancing the body's natural defense antioxidant system. Additionally, it exerts an anti-inflammatory impact by modulating *NF-κB* gene expression.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: The Scientific Committee of Mustansiriyah University's College of Pharmacy, Pharmacology and Toxicology Department granted ethical approval for this study (approval number: 24, reference number: 102, date: 28 October 2023).

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text.

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