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ORIGINAL RESEARCH

Hepatoprotective Effects of Cilnidipine in Cholestatic Liver Disease: Role of FXR and NRF2 Signalling

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Background: Bile acid (BA) is a type of cholesterol derivative that has long been established for its crucial role in the breakdown and absorption of fat from food. Cholestasis occurs when the liver fails to transfer BAs to the intestines. Chronic cholestatic diseases can lead to liver cirrhosis.

Objective: Ursodeoxycholic acid (UDCA) treatment is ineffective for certain cholestatic diseases like benign recurrent intrahepatic cholestasis (BRIC), despite increasing the hydrophilic bile acid pool. Moreover, studies indicate that UDCA and other bile acids affect liver cell functions, such as biotransformation through CYP enzymes. In hepatitis B virus transgenic mice, a UDCA-rich diet promoted hepatocyte proliferation and tumor growth. Hepatologists advise against using UDCA in patients with severe obstructive cholangiopathies. Given the foregoing, new medications are required to treat these illnesses.

Methods: Twenty-four male Wistar albino rats were separated into three groups (8 rats for each group): negative control group I, positive control group II (ANIT-induced cholestasis), and treatment group III (Cil and ANIT). The mRNA and protein expression levels of FXR, small heterodimer partner (SHP), bile salt export pump (BSEP), nuclear factor erythroid 2-related factor 2 (NRF2), hepatocyte nuclear factor 1α (HNF1α), sirtuin 1 (SIRT1), NADPH dehydrogenase-quinone-1 (NQO-1), and heme oxygenase-1 (HO-1) were assessed post euthanasia. Additionally, other tissue oxidative stress markers were measured.

Results: Cil significantly increased the mRNA expression levels of FXR, SHP, BSEP, HNF1α, and NRF2 and the protein expression levels of FXR, BSEP, SIRT1, NQO-1, and HO-1 in the treatment group compared with those in the positive control group. Additionally, Cil decreased the oxidative stress level compared with that in the ANIT-treated group.

Conclusion: The results suggest that Cil effectively treats cholestasis by affecting the FXR signaling system and the NRF2 pathway.

Keywords: ANIT, cholestasis, cilnidipine, FXR, NRF2, oxidative stress

Introduction

Bile acid (BA) is a type of cholesterol derivative that has long been established for its crucial role in the breakdown and absorption of fat from food.[1,2] Cholestasis occurs when the liver fails to transfer BAs to the intestines. Chronic cholestatic diseases can lead to liver cirrhosis.[3]

Ursodeoxycholic acid (UDCA) remains the only known treatment for cholestatic liver disease. It functions by blocking cell death, protecting cells, modulating the immune system, and lowering cholesterol levels.[4,5] While human BA comprises 3% UDCA, this level can increase to 50%, with daily doses between 13 and 15 mg/kg.[6] Certain types of cholestatic diseases, including benign recurrent intrahepatic cholestasis (BRIC), do not respond to treatment with UDCA; nonetheless, the bile acid pool is significantly enriched with this hydrophilic bile acid.[7] Additionally, new experimental research has shown that UDCA and other bile acids influence a range of liver cell processes, including biotransformation, through cytochrome P450 (CYP) enzymes at both the transcriptional and post-transcriptional stages. It has been shown that UDCA specifically induces the CYP3A subfamily.[8–11] CYP3A is essential for drug metabolism and participates in the pre-systemic extraction of more

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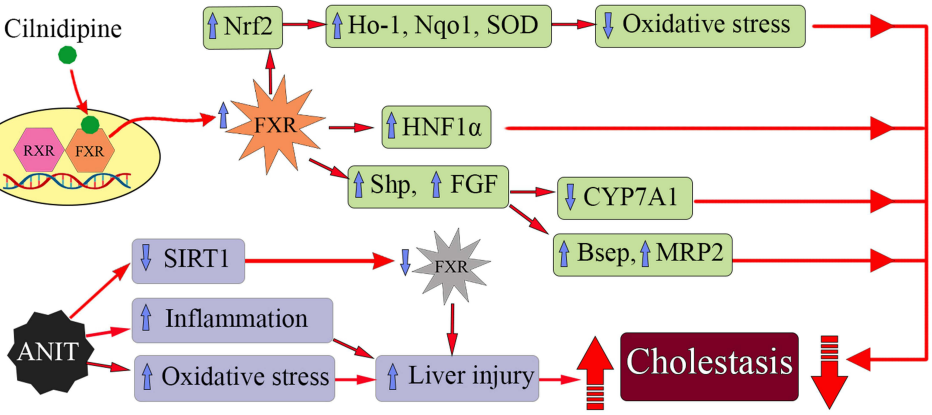
Graphical Abstract



than 50% of all available drugs.[12] Moreover, hepatocyte proliferation and tumor growth were stimulated in hepatitis B virus transgenic mice with a diet enriched with UDCA.[13] However, hepatologists generally believe that UDCA should be used very sparingly, or even not at all, in patients suspected of having cholangiopathies with a significant obstructive component, such as the late stages of primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), or biliary atresia.[14,15] Likewise, UDCA has only been used therapeutically for type-III biliary atresia (ductules larger than 50 μm).[16] Given the foregoing, new medications are required to treat these illnesses.

As the farnesoid X receptor (FXR) is a BA sensor, it can be targeted for the treatment of cholestasis.[17] FXR regulates fat metabolism (including its transport, synthesis, and absorption), whereas BAs are the endogenous ligands that activate this receptor.[18,19] BA also indirectly suppresses the expression of cytochrome P450 8B (CYP8B) and cytochrome P450 7A1 (CYP7A1), which are the enzymes involved in the synthesis of liver BAs. FXR also stimulates the transcription of a small heterodimer partner (SHP), which subsequently binds to liver receptor homolog-1 (LRH-1) and suppresses its function.[20] Additionally, FXR increases the expression of fibroblast growth factor-19 (FGF19), which in turn suppresses the expression of CYP7A1.[21,22] FXR also increases the expression of export transporters of hepatic BAs, including the bile salt export pump (BSEP) and multidrug resistance-associated protein 2 (MRP2).[23,24]

Another type of transporter affected by FXR is sodium taurocholate cotransporting polypeptide (NTCP), the primary protein governing BA absorption by the liver. However, the performance of this transporter is hindered by the activation of the FXR/SHP pathway.[25]

Currently, calcium channel blockers (CCBs), such as dihydropyridines and nondihydropyridines, are utilized for the treatment of hypertension.[26] Cilnidipine (Cil) is a recently discovered CCB that inhibits both L- and N-type channels. As N-type channels are located mainly in nerves and the brain, Cil is expected to affect nerve activity, which includes the inhibition of the sympathetic nervous system.[27] Recently, Cil was identified as an FXR agonist.[28]

1-α Naphthyl isothiocyanate (ANIT) has been used as a model chemical to study mechanisms of intrahepatic cholestasis for over two decades. When acutely delivered to rats and mice, ANIT causes bile ductular epithelial cell necrosis, which is followed by bile stasis, and bile duct proliferation.[29–31]

In hepatocytes, ANIT is detoxified via conjugation with glutathione (GSH). Multidrug resistance-associated protein 2 (Mrp2) transports the ANIT-GSH compound into bile, where ANIT dissociates from GSH. The ANIT that is released preferentially destroys bile-duct epithelial cells, resulting in cholangitis and eventual intrahepatic cholestasis.[32,33] Markers for cholestasis and liver injury, such as serum bile acids, ALT, and bilirubin were elevated 24 hours after ANIT treatment and peaked at 48 hours.[34]

Materials and Methods

Materials

The alpha-naphthyl isothiocyanate (ANIT) utilized for this study was acquired from Sigma Aldrich, USA, while the Cil (CAS Number: 132203-70-4) was acquired from Bide Pharmatech Limited of China. Biochemical indicator kits for glutathione peroxidase (GPx-1), malondialdehyde (MDA), and superoxide dismutase (SOD) were obtained from Wuhan USCN Business Co., Ltd./USCN Life Science KIT INC, China. The PCR primers purchased from Macrogen, Inc., South Korea, are listed in Table 1. Other PCR materials, including reverse transcription kits (RTKs) and quantitative polymerase chain reaction (qPCR) master mix, were acquired from Promega Corporation, USA. The Western blot materials used were acquired from Elabscience, USA. The antibodies used for protein quantification, as well as their manufacturers, are listed in Table 2.

Experimental Protocol

The animal care and study protocol for this undertaking was validated by the Local Study Ethics Committee of the College of Pharmacy at the University of Baghdad in Iraq. To acclimatize the animals, they were placed in a standard laboratory setting, which covered the elements of temperature (25±2°C) and illumination (12:12 h dark/light cycle).

The rats were randomly separated into three groups comprising eight rats each. Group I (negative control) was orally administered 1 ml/kg corn oil two days before euthanasia; Group II (positive control) was administered a single oral dose of 100 mg/kg ANIT two days before euthanasia;[35] Group III (treatment group) was orally administered 10 mg/kg Cil for seven days,[36] and 100 mg/kg ANIT two days before euthanasia, on Day 5. Upon the completion of this week-long exercise, the rats were euthanized via diethyl ether and cervical dislocation, and their liver tissues were excised into four sections: one was maintained in TRIzol reagent for subsequent RNA extraction, while the other was homogenized with

Table 1 The PCR Primers

Gene	Forward 5' →3'	Reverse 5' →3'
FXR	AGGCCATGTTCCTTCGTTC	CAGCTCCCCGACACTTTTATAG
SHP	GAGTCTTTCTGGAGCCTTGAG	AGGACTTCACACAATGCCC
NRF2	GCTATTTTCCATTCCCGAGTTAC	ATTGCTGTCCATCTCTGTCAG
HNF1α	AGAGTCCCTTCATGGCAAC	CTGAGGTTGGTGTCTGTGATC
BSEP	CAACGCATTGCTATTGCTCG	GTTCTGGATGGTGGACAAACG
GAPDH	TTCCAGGAGCGAGATCCCGCTAAC	CATGAGCCCTTCCACGATGCCAAAG

Table 2 Primary and Secondary Antibodies Used and Their Manufacturers

Target Protein	Species	Dilution	Manufacturers
FXR	Rabbit	1:5000	MyBioSource/USA
BSEP	Rabbit	1:5000	MyBioSource/USA
SIRT1	Rabbit	1:500	Elabscience/USA
NQO-1	Rabbit	1:500	Elabscience/USA
HO-1	Rabbit	1:1000	Elabscience/USA
Rabbit IgG	Goat Anti-Rabbit	1:10000	Elabscience/USA
Beta-actin	Rabbit	1:5000	Elabscience/USA

RIPA lysis buffer for subsequent protein extraction and quantification. In the other two sections, one was fixed with PBS-buffered formalin (10%) for later assessment of histopathological changes, and the other section was homogenized and used to measure oxidative stress markers.

Determination of Oxidative Stress Levels

After the animals were euthanized, a segment of liver tissue was removed and rinsed with phosphate-buffered saline (PBS). Subsequently, 1 g of liver tissue was homogenized with 9 ml of PBS. The homogenate was subsequently centrifuged for 10 minutes at low temperature, and the supernatant was collected for the assessment of superoxide dismutase (SOD), glutathione peroxidase-1 (GPx-1), and malondialdehyde (MDA) contents.

Quantitative Polymerase Chain Reaction (qPCR)

The rats' liver tissues were treated with TRIzol reagent, and RNA was extracted according to the directions provided by the manufacturer.[37] A spectrophotometer was used to measure the total RNA content and purity at 260 and 280 nm. Two micrograms of RNA was subsequently used to generate complementary DNA (cDNA) via reverse transcription via GoScript™ Reverse Transcriptase. The cDNA was stored at −80°C for subsequent PCR experiments. Bio-Rad® real-time PCR was then used in the RNA amplification process. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was used to standardize the quantity of the messenger ribonucleic acid (mRNA), while the $2^{-\Delta\Delta CT}$ method was used to generate all comparative data. The primers used for these experiments are listed in Table 1.

Western Blot Analysis

A mixture comprising 300 mg of liver tissue, 1 mL of RIPA lysis buffer, 10 µL of sodium orthovanadate (Na3VO4), and 10 µL of phenylmethylsulfonyl fluoride (PMSF) was used for the extraction of proteins from the accumulated liver tissues. The use of an Elabscience® BCA protein assay kit to measure the protein concentrations was followed by the placement of 10 µL of the SDS-protein mixtures of each sample into the wells of the preprepared SDS–PAGE gel. The separated proteins were then transferred to polyvinylidene difluoride (PVDF) membranes, which were then incubated in 5% skim milk at standard laboratory temperature for 90 minutes and constantly agitated. The PVDF membranes were then incubated in a refrigerator overnight while being agitated with primary antibodies against FXR, BSEP, sirtuin 1 (SIRT1), heme oxygenase-1 (HO-1), NAD(P)H dehydrogenase-quinone-1 (NQO-1), and beta-actin. A secondary antibody solution was then added to the PVDF membranes prior to their incubation for an additional 60 minutes on a shaker. The surfaces of the PVDF membranes were subsequently treated with the ECL working solution for a period of between one and five minutes. To complete this exercise, the intensity of protein expression was assessed via the Bio-Rad® ChemiDoc MP Imaging System, and the band images were evaluated via the ImageJ program.

Histological Examination

A piece of rat liver from each group was fixed with PBS-buffered formalin (10%) at 4°C for one week for histopathological evaluation. To detect hepatocellular necrosis, liver slices treated with formalin were sliced into 5 µm sections, stained with HE, and then examined under a microscope.[38,39]

A histopathologist scored the severity of liver histopathological changes as follows: score (-) refers to no pathological lesion; score (-/+) refers to very mild changes; score (+) refers to histopathological changes in <20% of the field; score (++) refers to histopathological changes in 20–60% of the field; and score (+++) refers to histopathological changes in >60% of the field.

Statistical Analysis

The data are presented as the means ± SDs. One-way analysis of variance was applied to determine the statistical significance, using GraphPad® Prism 9.5.1, with post hoc/Tukey testing for group comparisons. $P<0.05$ indicated significance.[40]

Results

Impact of Cilnidipine (Cil) on the Nuclear Factor Erythroid 2-Related Factor 2 (NRF2) Signaling Pathway

Many cholestatic ailments are associated with the encroachment of inflammatory cells, promoting abnormal BA secretion, blockage of bile ducts, and induction of cell death. Nuclear factor erythroid 2-related factor 2 (NRF2), a redox-sensitive transcription factor, is involved in several anti-inflammatory mechanisms. As shown in Figure 1, Group III delivered significantly higher levels of NRF2 mRNA than Groups I and II did.

Cholestatic liver damage is characterized by oxidative stress. The levels of GPX1, MDA, and SOD in liver tissue homogenates were measured. The outcomes are depicted in Figure 2. Compared with the control group, ANIT- and Cil-pretreated rats presented substantially lower tissue levels of GPX1 and SOD; however, the Cil pretreatment group presented higher levels than did the ANIT group (Figure 2A and B).

Compared with control rats, ANIT-treated rats presented a substantial increase in the level of tissue MDA, which is a measure of oxidative stress. Figure 2C shows that, compared with the ANIT group, the Cil pretreatment group had a much lower level.

Impact of Cilnidipine (Cil) on the Farnesoid X Receptor (FXR) Signaling Pathway

Several studies have shown that BA employs a nuclear receptor signaling cascade to control CYP7A1, FXR, and SHP expression. FXR regulates both enterohepatic circulation and the accumulation of BAs within cells. As depicted in Figure 1A, Group II presented significantly lower FXR mRNA expression than Group I did, whereas FXR mRNA expression was restored in Group III. The protective effects of FXR activation in cholestatic diseases have also been reported in previous studies.

Additionally, while the expression of BSEP, SHP, and hepatocyte nuclear factor 1α (HNF1α) mRNA was significantly lower in Group II than in Group I, the expression of these genes was significantly greater in Group III (Figure 1B–D).

Impact of Cilnidipine (Cil) on Rat Bile Salt Export Pump (BSEP), Farnesoid X Receptor (FXR), NAD(P)H Dehydrogenase-Quinone-1 (NQO-1), Sirtuin 1 (SIRT-1), and Heme Oxygenase-1 (HO-1) Protein Levels

According to the Western blot analysis results, while ANIT decreased the protein levels of BSEP, FXR, NQO-1, SIRT1, and HO-1, these levels increased upon the administration of Cil (Figure 3).

Effects of Cilnidipine (Cil) on Liver Histopathology

The severity of liver damage was notably lower in the Cil-treated group (Group III) than in the ANIT-treated group (Group II) (Figure 4).

Discussion

The proliferation of bile ducts, the death of parenchymal cells, inflammation, and liver fibrosis are the clinical conditions that impede the flow of bile, culminating in cholestatic liver damage.[41] Endorsed by the United States Food and Drug Administration (USFDA) body, ursodeoxycholic acid (UDCA) is a drug used for the treatment of gallstones and primary biliary cirrhosis, as well as for the prevention of gallstone development, in obese individuals who shed weight rapidly.[42] However, as certain cholestatic ailments do not respond well to UDCA, the development of innovative alternatives ought to be in the pipeline.

FXR plays a crucial role in several aspects of inflammation and metabolism, making it a desirable therapeutic target. FXR ligands are effective in treating nonalcoholic steatohepatitis (NASH), diabetes, and primary biliary cholangitis (PBC) by improving bile acid, glucose, and lipid metabolism and reducing inflammation.[43,44] However, concerns concerning safety and long-term clinical outcomes have been raised. These may include severe itching, a decrease in high-density lipoprotein cholesterol, and an unfavorable serum lipid profile with raised total and low-density lipoprotein

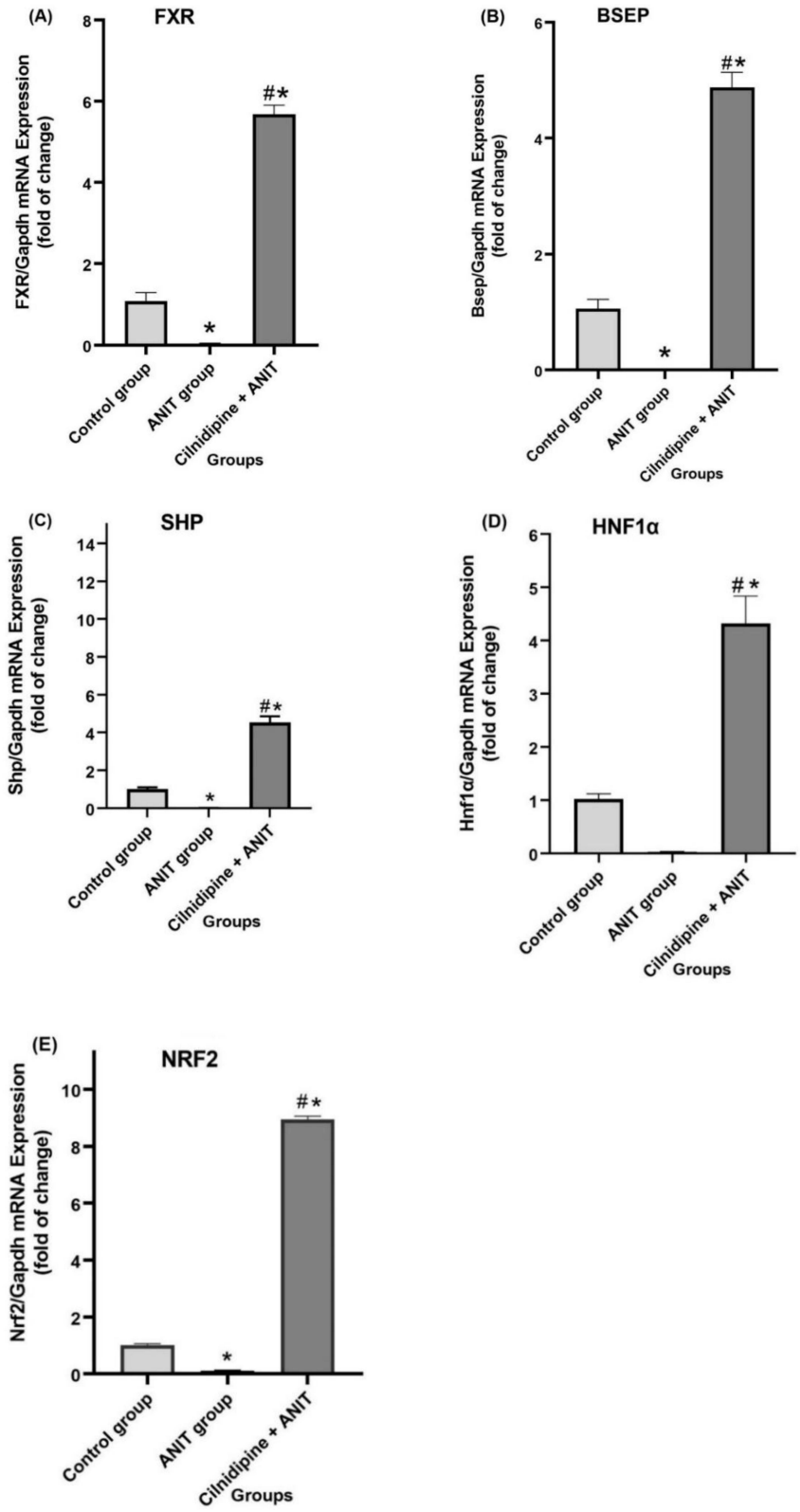


Figure 1 Impact of Cil on BA gene expression in ANIT-induced cholestasis in rats. The gene expression levels of (**A**) FXR, (**B**) BESP, (**C**) SHP, (**D**) HNF1α, and (**E**) NRF2 were measured via real-time PCR. Each value is represented as the mean ± SD; *P<0.05 vs Group I; #P<0.05 vs Group II.

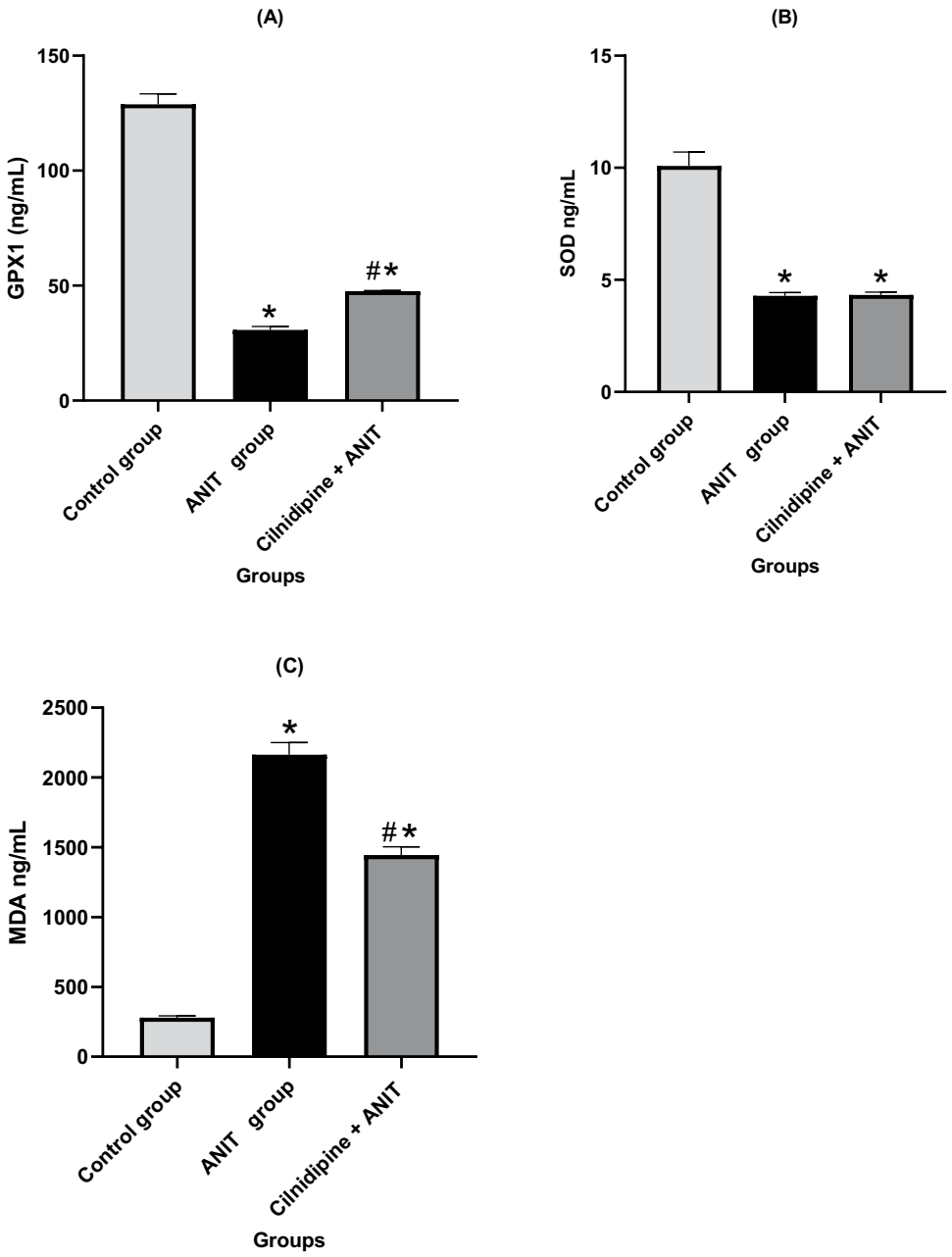


Figure 2 The effects of Cil on tissue GPx-1 (**A**), MAD (**B**), and SOD (**C**) in rats with cholestatic liver damage produced by ANIT. Each value is presented as the mean ± SD; *P < 0.05 vs the control group; #P < 0.05 vs the ANIT-treated group.

cholesterol.[45,46] Because it preserves the integrity of the intestinal barrier and prevents bacterial translocation, FXR is a promising target for the treatment of inflammatory bowel disease (IBD).[47] Obeticholic acid (OCA), an FXR agonist, was recently approved for use in combination with UDCA to treat PBC, despite data showing that OCA had side effects that worsen biliary damage in animal models of obstructive cholestasis.[48,49] After three years of follow-up, 33% of patients with primary biliary cholangitis on OCA reported fatigue, and up to 77% had pruritus.[50] On the other hand, nonsteroidal FXR agonists are being developed and studied in hopes of offering similar therapeutic benefits with fewer side effects. They might have a better safety record, particularly in relation to hepatotoxicity, a serious issue with steroid-based FXR agonists.[51]

The binding of the FXR-RXR heterodimer complex to BAs activates the transcriptional repressor SHP, which consequently downregulates the expression of CYP7A1 and CYP8B1, the two main enzymes involved in the synthesis

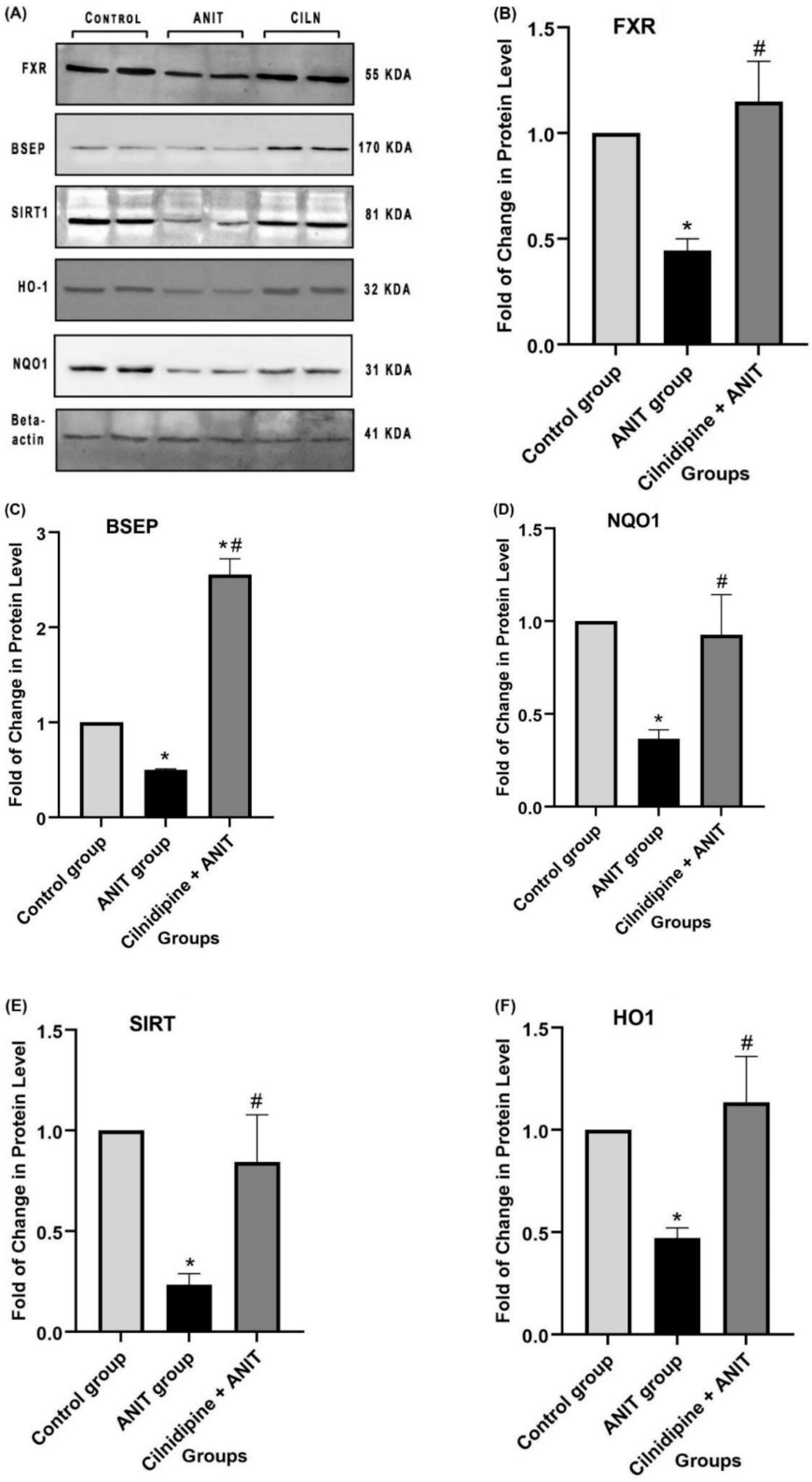


Figure 3 Impact of Cil on the mRNA expression of BSEP, FXR, NQO-1, SIRT1, and HO-1 in rats with ANIT-induced cholestatic disease. (**A**) Western blot images of FXR, BSEP, SIRT1, H0-1, NQO-1, and beta-actin. Folds of change in protein levels with respect to the beta-actin of BSEP, FXR, NQO-1, SIRT1, and HO-1 are shown (**B–F**), respectively. Each value is represented as the mean ± SD (n=8); *P<0.05 vs Group I; #P<0.05 vs Group II.

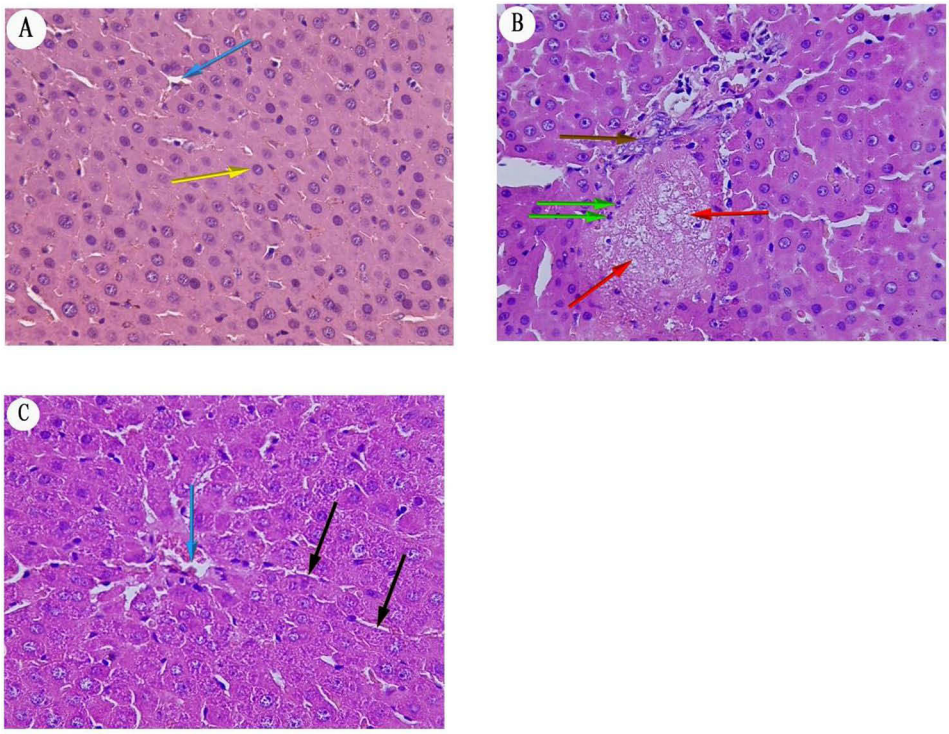


Figure 4 Histopathological changes. The liver section in the control group (**A**) had a normal histological structure characterized by a central vein (shown by a blue arrow) surrounded by strands of hepatocyte cells (indicated by a yellow arrow). Its score is (0). In the ANIT-treated group (**B**), the slice exhibited a focal necrotic region (shown by the red arrow); there was also a proliferation of bile ducts (indicated by the brown arrow) accompanied by infiltration of inflammatory cells (indicated by the green arrow), and its score was (++). Histologically, the cilnidipine-treated Group III (**C**) slice looks normal, except for a little sinusoid dilatation (black arrow). The central vein (shown by a blue arrow), its score was (-/+).

of BAs. This occurrence also prompts the expression of BSEP.[52,53] As shown in Figures 1 and 3, Cil upregulated the mRNA and protein expression of FXR while downregulating signaling cascades. Thus, Cil significantly increases the gene expression levels of FXR, SHP, and BSEP, as well as the protein expression levels of FXR and BSEP.

The liver, pancreas, kidneys, and digestive tract all express HNF1α.[54] Among the genes regulated by HNF1α in the liver are β-fibrinogen, albumin, and α1-antitrypsin. Null mice without the T-cell factor-1 transcription factor (Tcf1-/-) were used to examine the role of HNF1α in terms of balancing the cholesterol level. These animals presented impaired high-density lipoprotein (HDL) metabolism, aberrant BA transport, and increased BA and cholesterol synthesis.[55] Bile acids (BAs) suppress HNF1α by reducing the activity of HNF4α.[56] As shown in Figure 1D, the mRNA expression levels of HNF1α are significantly higher in Group III, while reduced in Group II.

Under cholestatic conditions, oxidative stress escalates liver damage, contributing greatly to the death of hepatocytes. When hepatocytes and mitochondria in the liver are subjected to apoptotic BAs, they produce reactive oxygen species (ROS).[57] The transcription factor NRF2 plays a role in the detection of oxidative stress in cells. Kelch-like Ech-associated protein 1 (Keap1) facilitates the retention of NRF2 in the cytosol. The release and nuclear translocation of NRF2 occur when oxidative stress induces changes in the sulfhydryl groups of Keap1.[58,59]

The functions of Nrf2 and FXR in regulating oxidative stress and inflammation in the liver are interrelated. The FXR preserves BA homeostasis and guards against BA-induced oxidative stress, whereas Nrf2 is mainly concerned with detoxifying ROS and lowering oxidative damage. The prevention of inflammation and the progression of chronic liver disorders depend on the coordinated regulation of Nrf2 and the FXR.

As previously stated, Nrf2 activation in the liver improves lipid profiles and lowers the buildup of BA. Mice studies have demonstrated that Nrf2 activation reduces liver BA concentrations, most likely as a result of less hepatic BA production.[60] Examining how the Nrf2 and FXR pathways interact is vital because the FXR is key to BA metabolism. Studies reveal that this relationship is mediated by proteins like β-catenin and P300.[61] Activated FXR promotes P300

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binding to Nrf2 by reducing FXR binding to ß-catenin and facilitating transcription of downstream genes. These results point to a tight connection between the Nrf2, FXR, P300, and β-catenin signaling pathways, which are essential for preserving BA homeostasis.[62]

The activation of NRF2 batteries serves to reduce the oxidative load in cells by increasing the glutathione (GSH), NQO-1, HO-1, and catalase concentrations while lowering the reactive intermediates, thereby enhancing the overall chemical detoxification of cells via phase II conjugation.[63,64]

As depicted in Figures 1 and 3, in comparison with Group II, Group III presented considerably higher mRNA expression levels of NRF2 and higher protein expression levels of NQO-1 and HO-1.

Consistent with earlier results, α-naphthyl isothiocyanate (ANIT) significantly increased the MDA level but decreased the GPx-1 and SOD levels in the cholestatic model group (Figure 2).[65] However, Cil pretreatment antagonized these effects.

In summary, SIRT1 activators impede the cytoplasmic breakdown and acetylation of FXR. Effective treatments for acute and chronic liver damage are achieved through a combination of SIRT1 activators and FXR agonists.[66] As shown in Figure 3E, in Group II, while the protein expression levels of SIRT1 were significantly decreased, these levels were restored following pretreatment with Cil.

Conclusion

The results showed that Cil significantly increased the mRNA expression levels of farnesoid X receptor (FXR), small heterodimer partner (SHP), bile salt export pump (BSEP), hepatocyte nuclear factor 1α (HNF1α), and nuclear factor erythroid 2-related factor 2 (NRF2), as well as the protein expression levels of FXR, BSEP, sirtuin 1 (SIRT1), NAD(P)H dehydrogenase-quinone-1 (NQO-1), and heme oxygenase-1 (HO-1) compared to the ANIT-treated group. Additionally, Cil decreased oxidative stress markers compared to the ANIT group. The findings suggest that Cil effectively alleviates cholestasis by influencing the FXR signaling pathway and the NRF2 pathway, indicating its potential as a new drug for treating cholestatic liver diseases that do not respond well to ursodeoxycholic acid (UDCA).

Abbreviations

ANIT, Alpha-naphthyl isothiocyanate; BA, Bile acid; BRIC, Benign recurrent intrahepatic cholestasis; BSEP, Bile salt export pump; CCBs, Calcium channel blockers; cDNA, Complementary DNA; Cil, Cilnidipine; CYP7A1, Cytochrome P450 7A1; CYP8B, Cytochrome P450 8B; FGF19, Fibroblast growth factor-19; FXR, Farnesoid X receptor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; GSH, Glutathione; GPx-1, glutathione peroxidase; HDL, High-density lipoprotein; HNF1α, Hepatocyte nuclear factor 1α; HO-1, Heme oxygenase-1; Keap1, Kelch-like Ech-associated protein 1; LRH-1, Liver receptor homolog-1; MDA, Malondialdehyde; mRNA, Messenger ribonucleic acid; Na3VO4, Sodium orthovanadate; NQO-1, NAD(P)H dehydrogenase-quinone-1; NRF2, Nuclear factor erythroid 2-related factor 2; NTCP, Sodium taurocholate cotransporting polypeptide; PMSF, Phenylmethylsulphonyl fluoride; PVDF, Polyvinylidene difluoride; qPCR, Quantitative polymerase chain reaction; ROS, Reactive oxygen species; RTK, Reverse transcription kits; SHP, Small heterodimer partner; SIRT1, Sirtuin 1; SOD, Superoxide dismutase; Tcf1-/-, T-cell factor-1 transcription factor; UDCA, Ursodeoxycholic acid; USFDA, United States Food and Drug Administration.

Ethics Statements

The Animal Research Local Ethics Committee University of the College of Pharmacy at the University of Baghdad approved this study on 20 April 2023 (Approval Number: RECAUBCP). The authors declare their responsibility for the application of these ethics as reported in the NIH Guide for the Care and Use of Laboratory Animals.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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