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RESEARCH ARTICLE

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Effects of bicyclol on hepatic sinusoidal obstruction syndrome induced by *Gynura segetum*

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Abstract

Background: The intake of *Gynura segetum*, a traditional Chinese medicine, may be induce hepatic sinusoidal obstruction syndrome (HSOS). It has a high mortality rate based on the severity of the disease and the absence of therapeutic effectiveness. Therefore, the current study was designed to investigate the effects of bicyclol on HSOS induced by *Gynura segetum* and the potential molecular mechanisms.

Methods: *Gynura segetum* (30 g/kg) was administered for 4 weeks in the model group, while the bicyclol pretreatment group received bicyclol (200 mg/kg) administration. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol (CHO), triglyceride (TG), and liver histological assays were detected to assess HSOS. The gene expressions of cytochrome P450 (*CYP450*) isozymes were quantified by real-time PCR. Moreover, hepatocellular apoptosis was detected using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, then apoptosis and autophagy-related markers were determined using Western blot.

Results: As a result, bicyclol pretreatment is notably protected against *Gynura segetum*-induced HSOS, as observed by reducing serum ALT levels, inhibiting the reduction in CHO and TG levels, and alleviating the histopathological changes. Bicyclol pretreatment inhibited the changes in mRNA levels of *CYP450* isozymes (including the increase in *CYP2a5* and decrease in *CYP2b10*, *2c29*, *2c37*, *3a11*, and *7b1*). In addition, the upregulation of Bcl-2 and the downregulation of LC3-II/LC3-I proteins expression in HSOS were inhibited with bicyclol pretreatment.

Conclusion: Bicyclol exerted a protective effect against HSOS induced by *Gynura segetum*, which could be attributed to the regulated expressions of *CYP450* isozymes and alleviated the downregulation of autophagy.

KEYWORDS
autophagy, Bicyclol, cytochrome P450, *Gynura segetum*, HSOS

1 | INTRODUCTION

Hepatic sinusoidal obstruction syndrome (HSOS), also referred to as sinusoidal obstruction syndrome (SOS) or hepatic veno-occlusive disease (HVOD), is a non-thrombotic obstructive disease of the hepatic sinusoids characterized by the absence of thrombosis or other underlying hepatic venous disease.[1,2] The intake of special medicinal herbs, such as *Carpesii fructus*, *Lycopi herba*, is a primary etiology for HSOS due to the presence of the toxic substances known as pyrrolizidine alkaloids (PAs).[3,4] The major cause of HSOS in China is the ingestion of a PA-producing *Gynura segetum*, accounting for 50%–89% of reported HSOS cases.[5] However, the precise pathogenic mechanism of *Gynura segetum* causing HSOS is not clearly understood. Moreover, current therapeutic strategies for HSOS induced by PAs include cessation of exposure to PAs, symptomatic treatment, anticoagulant therapy, transjugular intrahepatic shunt (TIPS), and liver transplantation, which means that there is no definitive treatment for PA-induced HSOS.[6]

Bicyclol is an approved synthetic drug that has been widely used for the treatment of chronic viral hepatitis type B (HBV) and viral hepatitis type C (HCV) in China.[7] Previous studies have well documented that bicyclol exerts a protective effect against liver injuries in various experimental models with respect to several chemical and pharmaceutical toxins, ischemia–reperfusion and partial hepatectomy. This function is mediated by multiple mechanisms including antioxidant action, regulation of cytokine secretion, bidirectional regulation of expression of *CYP450*, inhibition of apoptosis and induction of cellular autophagy.[8–13] Our previous study reported that hepatocyte necrosis and autophagy occur in HSOS induced by *Gynura segetum*, while bicyclol has hepatoprotective and ameliorative effects on autophagy.[14] Taken together, bicyclol exerts hepatoprotective effects on various types of liver diseases via multipath signaling pathways, and may be used in the symptomatic treatment of HSOS induced by *Gynura segetum*; however, its effect related to HSOS or *Gynura segetum* has not been reported.

Therefore, the present study aimed to investigate the effects of bicyclol on HSOS induced by *Gynura segetum* and the potential molecular mechanisms.

2 | MATERIALS AND METHODS

This study was approved by the Experimental Animal Ethics Committee of Zhejiang Pharmaceutical College (Approval number: ZYLL202105019) and conducted in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.1 | *Gynura segetum* extract and drug

The roots of 1000 g *Gynura segetum* (acquired from Anhui Bozhou Pharmaceutical Co) were immersed in water for 2 h and boiled for 1.5 h, followed by filtration to collect filtrate A. The extracted roots were supplemented with 3000 ml of water, and the mixed decoction was boiled for 1.5 h as the above way, then filtered to obtain filtrate B. Finally, filtrates A and B were merged and boiled to reduce to a volume of 500 ml that was refrigerated and reserved.[15] Bicyclol (No.200910) was procured from the Beijing Union Pharmaceutical Company.

2.2 | Animals and treatment

Male ICR mice (22–24 g body weight, provided by the Zhejiang Academy of Medical Sciences) were used for the present study. A total of 30 mice were randomized into the control group, *Gynura segetum*-induced HSOS, and bicyclol-pretreated groups. The mice in the bicyclol-pretreated group were administered bicyclol (200 mg/kg, suspended in 10% carboxymethyl cellulose) intragastrically twice daily for 4 weeks, while the other two groups were administered an equivalent volume of the vehicle of bicyclol as control. After 1 h, a 30 g/kg concentrated decoction of *Gynura segetum* was administered by gavage in the *Gynura segetum*-induced HSOS group and bicyclol pretreated-groups, while an equivalent volume of distilled water was administered to the control group according to the same approach. At 4 weeks after administration, all mice were fasted for 12 h, and then the liver tissues and blood samples (harvested through the eyeball) were immediately gathered. The liver tissues were freeze-clamped by liquid nitrogen and stored at −80°C to extract the mRNA and protein from the liver tissues. Approximately, 200 mg of liver tissues were fixed in 10% formaldehyde prior to tissue freezing for histopathology. The plasma was collected from the blood samples by centrifugation at 3000*g*, 4°C for 10 min, and the content of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol (CHO), and triglyceride (TG) was determined.

2.3 | Biochemistry analyses

Serum ALT, AST, CHO, and TG levels were, respectively, quantified using the corresponding assay kits (Ningbo Meikang Biological Co) on a biochemical analyzer (PUZS-300, Beijing Prolong New Technology Co), following the standard protocols.

2.4 | Histopathology

After mice were executed, liver tissues were fixed in 10% neutral formalin solution, then embedded in paraffin, sectioned at 5-μm-thick sections, and stained with hematoxylin–eosin (H&E) and Masson for morphological observation. The pathology was assessed using a modified scoring system,[16] involving six parameters: sinusoidal hemorrhage, central venous subendothelial hemorrhage, hepatocyte-coagulative necrosis, central venous endothelial damage, central venous subendothelial fibrosis, and sinusoidal fibrosis. All sections were viewed under a light microscope (Olympus), and

the images were captured at ×100 magnification. Scoring system of pathology is evaluated by a pathologist based on the number of corresponding cells or pathological structures in the field of view at the same magnification of the microscope. Liver fibrosis was evaluated by Masson's staining and quantified by ImageJ software.

2.5 | Real-time polymerase chain reaction (RT-PCR) assay

Total RNA was extracted from mice liver samples using TRIzol reagent (Vazyme Biotech Co., Ltd). The cDNA was produced using a total of 1 μg of RNA with the HiScript III All-in-one RT Reagent kit (Vazyme Biotech Co., Ltd). Finally, the gene expression was examined using the ChamQ SYBR Mixture real-time detection system. The reaction mixture in a volume of 20 μl as follows: cDNA (2 μl), ChamQ SYBR Mixture (10 μl, Vazyme Biotech), and 0.5 μl of each primer (10 pM). The amplification conditions were as follows: 95°C (30 s), followed by 40 cycles of 95°C (10 s) and 60°C (30 s). The primers are listed in Table 1.

2.6 | TUNEL assay

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay was utilized to detect liver tissues with a situ cell apoptosis detection kit (Wuhan Boster Biological Engineering Co. Wuhan). The paraffin-embedded tissue sections were pretreated with proteinase K for 15 min and incubated with TdT and DIG-d-UTP labeling buffer at 37°C in a humidified chamber for 2 h. Next, the sections were flushed with a Tris-buffered saline (TBS) buffer for 2 min and incubated in a blocking buffer at 37°C for 30 min. Then, the sections were incubated in biotinylated anti-digoxin antibodies (1:100 dilution) at 37°C for 40 min and rinsed with TBS buffer three times for 2 min. Subsequently, streptavidin-fluorescein (FITC) was used to stain the sections that were then rinsed with TBS buffer for 5 min, five times. Ultimately, the sections were placed in an antifading solution and examined under a fluorescence microscope. The positive cells with green color were quantified by ImageJ software.

2.7 | Protein extraction and Western blot assay

Liver tissues of mice in every group were lysed with radioimmunoprecipitation (RIPA) buffer (Beyotime Institute of Biotechnology). The supernatants were then collected by centrifugation at 13,000 rpm for 15 min at 4°C. Next, protein concentrations were detected with the BCA protein assay kit (TianGen Biotech Co). A western blot was conducted using total protein (10–50 μg). Primary antibodies including anti-Caspase-3, B cell lymphoma/leukemia (Bcl)-2 (Bcl-2), LC3, autophagy-related 12 (Atg12), and β-actin were detected, and corresponding horseradish peroxidase-conjugated anti-rabbit or anti-goat were employed as secondary antibodies (all antibodies were from ProteinTech Group, Inc). Ultimately, the specific protein bands were visualized using an ECL Western Blot Detection System (LAS 400 Mini, General Electric Company, Boston).

2.8 | Statistical analysis

All data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) by Graph Pad Prism 9.0 software. The differences between means were analyzed by Student–Newman–Keuls (SNK) test for multiple comparisons. Statistical significance was set at $p < 0.05$.

TABLE 1 Polymerase chain reaction primer sets in real-time PCR.

Gene	Primer sequences	GenBank™ accession no.
CYP2A5	Forward TGTAGTCAGCACCAAGTTC Reverse CTCCTTCCTCATCCGAATG	NM_007812
CYP2B10	Forward GGCACTCCAATAGGTATAAGA Reverse CAGTCATCCACGAGATTCA	NM_009999
CYP2C29	Forward GCTTCCTTCACTGCTTCA Reverse GCCAATCCTTCACCAACT	NM_007815
CYP2C37	Forward CTTCACTGCCTCATATCCAT Reverse GACATCTGCCAATCCTTCA	NM_010001
CYP3A11	Forward CTCTCAAGTCTATTAGCAATGG Reverse AGATGGAATACCTGGATATGG	NM_007818
CYP7B1	Forward TCTGTGTTCCAATCTGTGAT Reverse GCTTACTGATGACGACCTT	NM_007825

Abbreviations: *CYP2A5*, cytochrome P450, family 2, subfamily a, polypeptide 5; *CYP2B10*, cytochrome P450, family 2, subfamily b, polypeptide 10; *CYP2C29*, cytochrome P450, family 2, subfamily c, polypeptide 29; *CYP2C37*, cytochrome P450, family 2, subfamily c, polypeptide 37; *CYP3A11*, cytochrome P450, family 3, subfamily a, polypeptide 11; *CYP7B1*, cytochrome P450, family 7, subfamily b, polypeptide 1.

3 | RESULTS

3.1 | Effect of bicyclol on the changes of serum biochemical indicators in HSOS mice

As illustrated in Figure 1, serum ALT and AST levels in the HSOS group were increased to 8.6- and 2.3-fold of the control group, respectively. After bicyclol pretreatment, the level of serum ALT in the HSOS group declined remarkably, while the decrease in AST level was not statistically significant. In addition, compared to the control group, serum CHO and TG levels in the HSOS group were decreased to 64.7% and 29.0%, and bicyclol pretreatment can significantly ameliorate the reduction in serum CHO and TG levels (Figure 1C,D).

3.2 | Effects of bicyclol on the changes in liver histopathology in HSOS mice

To directly evaluate the effect of bicyclol on HSOS, H&E, and Masson's staining of the liver tissues were performed. As shown in Figures 2,3, compared to the control group, the liver histopathological changes in the *Gynura segetum*-induced HSOS group show central venous endothelial damage and hemorrhage, severe sinusoidal congestion and space narrowing, coagulative necrosis of hepatocytes, inflammatory cell accumulation and fibrosis in portal areas, some hepatocyte nucleus pyknosis, fatty degeneration of hepatocytes, and liver lobule structure destruction. The pretreatment with bicyclol relieved the above histopathological changes induced by *Gynura segetum*. The pathological evaluation of every group is shown in Table 2.

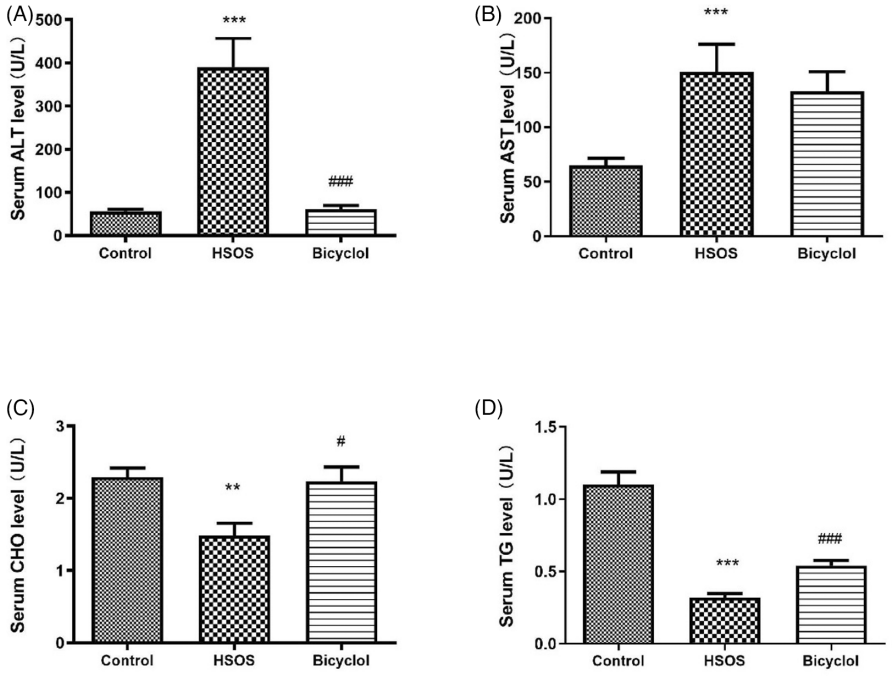

FIGURE 1 Effect of bicyclol on the increase in serum transaminases and serum lipids in HSOS mice. Samples of blood were collected at 4 weeks after *Gynura segetum* administration: (A) serum ALT; (B) serum AST; (C) serum CHO; (D) serum TG. $^{**}p < 0.01$ versus control group, $^{***}p < 0.001$ versus control group, $^{\#}p < 0.05$ versus HSOS group, $^{\#\#\#}p < 0.001$ versus HSOS group.

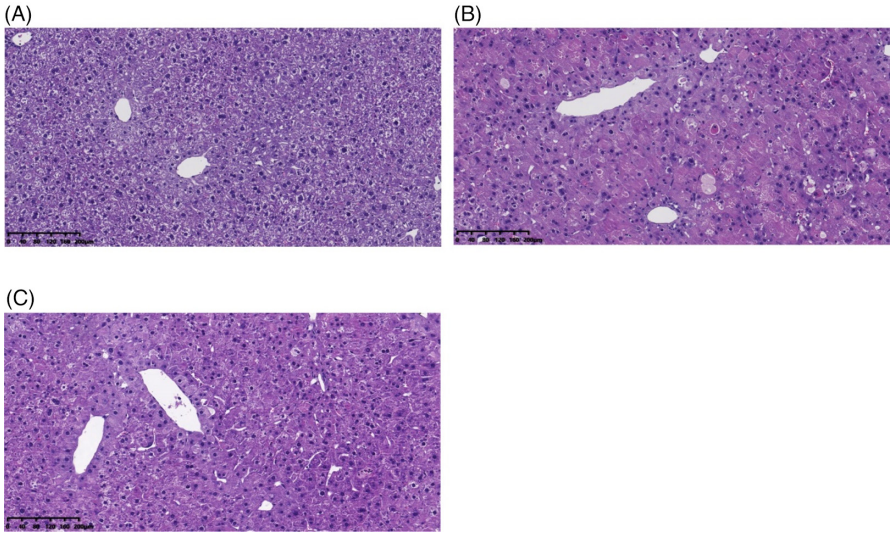

FIGURE 2 Effects of bicyclol on liver histopathology in HSOS mice by H&E staining. In each group, liver specimens were gathered after 4 weeks of treatment. (A) control group; (B) HSOS group. (C) Bicyclol-pretreated group. Original magnification, ×100.

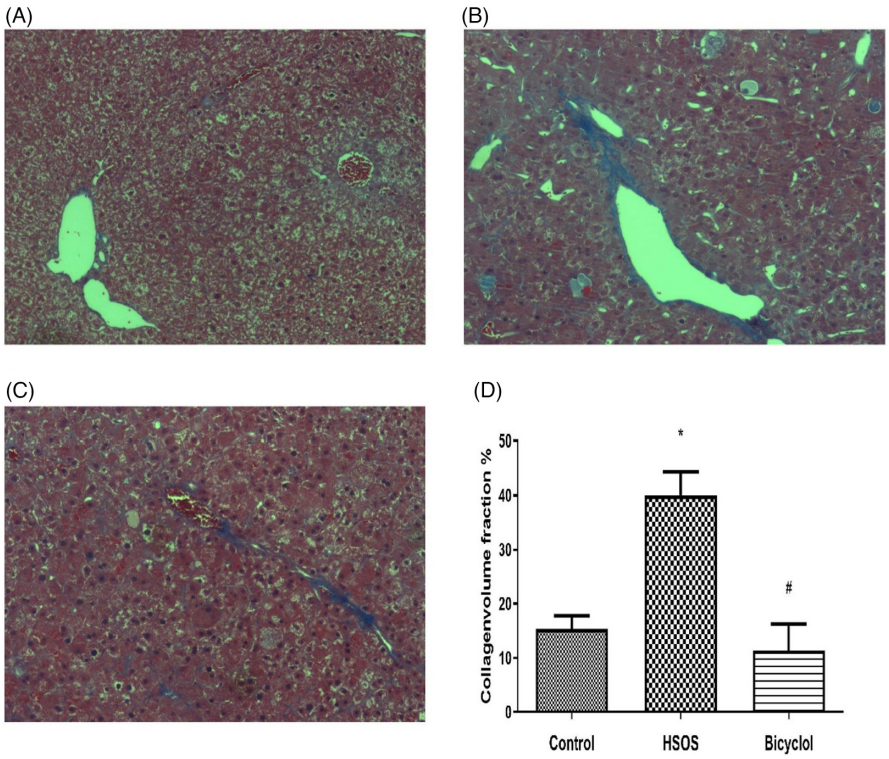

FIGURE 3 Effects of bicyclol on liver histopathology in HSOS mice by Masson staining. Blue represents collagen while red represents muscle fibers. (A) control group; (B) HSOS group. (C) Bicyclol-pretreated group. (D) Collagen volume fraction % of every group. Original magnification, ×100. $^{*}p < 0.05$ versus control group, $^{\#}p < 0.05$ versus HSOS group.

TABLE 2 H&E staining evaluation of the effect of bicyclol on liver histopathology in *Gynura segetum*-induced HSOS mice.

Parameters	Control group	HSOS model group	Bicyclol group
Central venous endothelial damage and hemorrhage	–	+++	++
Sinusoidal hemorrhage	–	+++	++
Hepatocyte coagulative necrosis	–	+++	++
Fibrosis in portal areas	–	+++	++
Liver lobule structure	normal	Structure disappeared	Presence

Note: "-" represents none of corresponding cells or pathological structures; "+" represents 1–3 of corresponding cells or pathological structures; "++" represents 3–6 of corresponding cells or pathological structures; "+++" represents 7–10 of corresponding cells or pathological structures.

3.3 | Effects of bicyclol on mRNA expressions of *CYP* isozymes in the liver tissues of HSOS mice

As illustrated in Figure 4, the mRNA expression levels of *CYP2b10*, *2c29*, *2c37*, *3a11*, and *7b1* in the HSOS group were obviously declined to 29.8%, 6.2%, 16.9%, 54.8%, and 0.5% compared to the control group, respectively, and the decreasing trend was significantly attenuated by bicyclol pretreatment. Additionally, compared to the control group, *CYP2a5* mRNA expression was upregulated 25-fold in the HSOS group, which was notably downregulated by bicyclol pretreatment.

3.4 | Effect of bicyclol on the hepatic apoptosis in HSOS mice

In this study, the TUNEL assay was performed to investigate whether apoptosis is involved in HSOS. As illustrated in Figure 5, the proportion of apoptotic cells in the liver tissues was higher in the HSOS group than in the control group. Moreover, the proportion was decreased but not significantly by bicyclol pretreatment.

3.5 | Effect of bicyclol on the expression levels of caspase-3 and Bcl-2 proteins in the liver tissues of HSOS mice

To further study the function of bicyclol on the hepatocyte apoptosis of HSOS, apoptosis marker proteins, including caspase-3 and Bcl-2, were detected. As illustrated in Figure 6, the protein levels of caspase-3 and Bcl-2 in the liver tissues were notably upregulated in the HSOS group compared to the control group. The upregulation of Bcl-2 was significantly inhibited by bicyclol pretreatment, while it alleviated the upregulation of caspase-3 although not significantly.

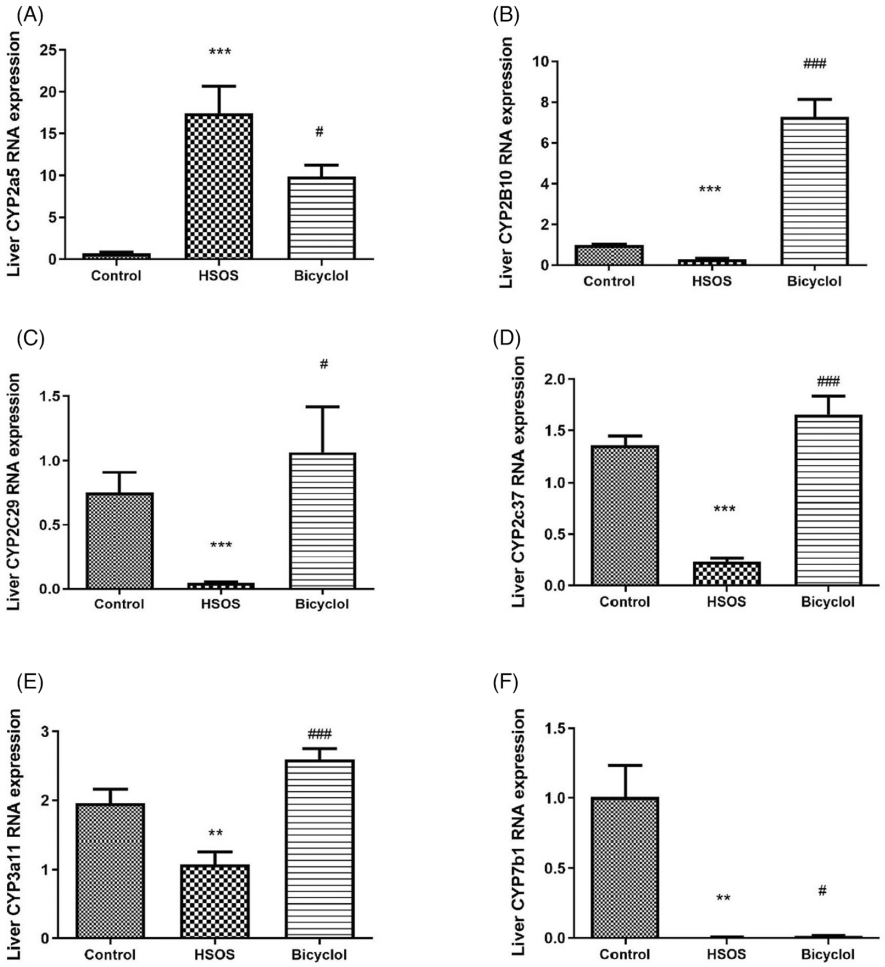

FIGURE 4 Effects of bicyclol on the mRNA expression of CYP isozymes in HSOS mice. (A) Effect of bicyclol on *CYP2a5* mRNA expression in HSOS mice; (B) Effect of bicyclol on *CYP2b10* mRNA expression in HSOS mice; (C) Effect of bicyclol on *CYP2c29* mRNA expression in HSOS mice; (D) Effect of bicyclol on *CYP2c37* mRNA expression in HSOS mice; (E) Effect of bicyclol on *CYP3a11* mRNA expression in HSOS mice; (F) Effect of bicyclol on *CYP7b1* mRNA expression in HSOS mice. $^{**}p < 0.01$ versus control group, $^{***}p < 0.001$ versus control group, $^{\#}p < 0.05$ versus HSOS group, $^{\#\#\#}p < 0.001$ versus HSOS group.

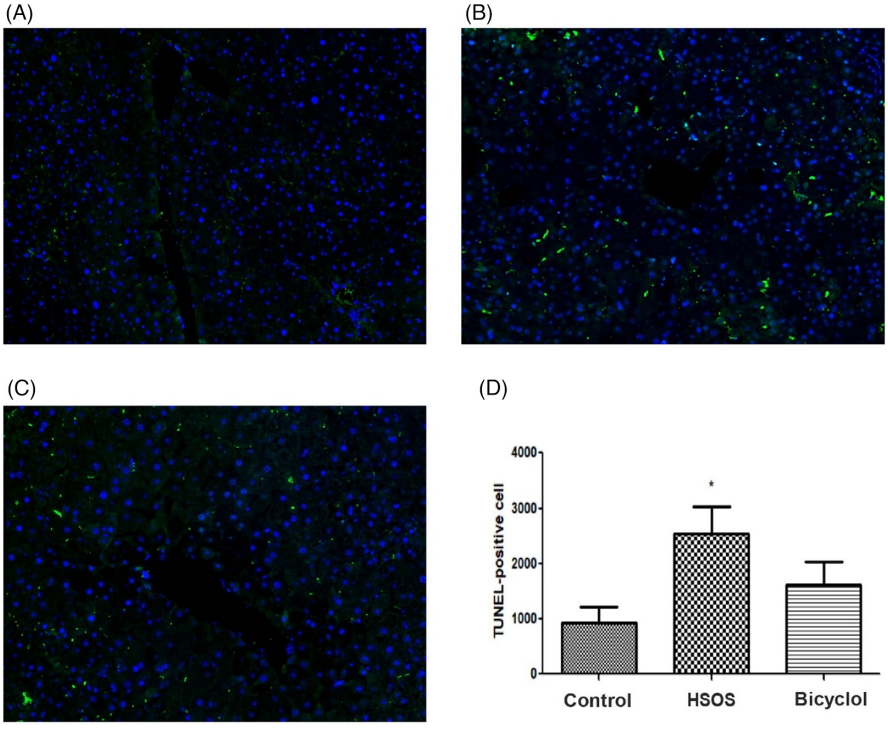

FIGURE 5 Effect of bicyclol on the increase of hepatic apoptosis in HSOS mice (TUNEL assay). Representative sections of TUNEL staining show apoptosis in mice from the Control, HSOS, and bicyclol-pretreated groups. Foci (green) were quantified using Image J software. (A) Control; (B) HSOS group; (C) Bicyclol-pretreated group; (D) TUNEL-positive cells in every group. Original magnification ×100. $^{*}p < 0.05$ versus control group.

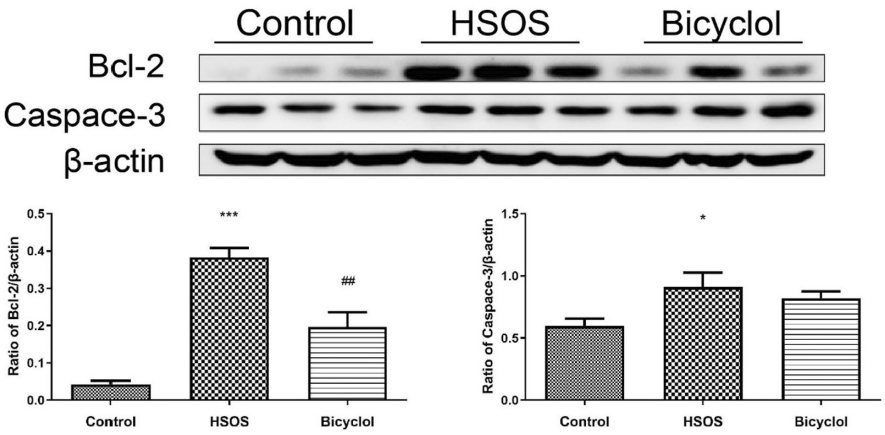

FIGURE 6 Effect of bicyclol on the protein expression levels of Caspase-3 and Bcl-2 in HSOS mice. Representative immunoblot images of the experiments are shown above, and the graphs below show the corresponding analysis. $^{*}p<0.05$ versus control group, $^{***}p<0.001$ versus control group, $^{\#\#}p<0.01$ versus HSOS group.

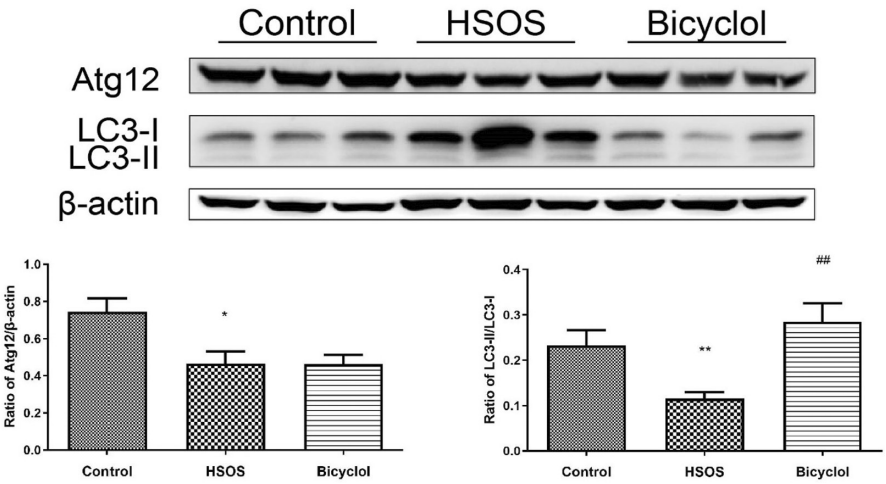

FIGURE 7 Effect of bicyclol on hepatic autophagy in HSOS mice. Autophagy proteins including LC3 and Atg12 were determined by Western blotting in the liver tissue of every group. Experimentally representative immunoblot images are illustrated above, and the corresponding analysis is shown in the graphs below. $^{*}p<0.05$ versus control group, $^{**}p<0.01$ versus control group, $^{\#\#}p<0.01$ versus HSOS group.

3.6 | Effect of bicyclol on the hepatic autophagy in HSOS mice

To evaluate the autophagy in *Gynura segetum*-induced HSOS mice, the markers of autophagy, LC3 and Atg12, were detected in the liver tissues. As illustrated in Figure 7, the levels of autophagy-related proteins (LC3-II/LC3-I and Atg12) were downregulated in the HSOS group compared to the control group. Furthermore, bicyclol pretreatment notably inhibited the downregulation of LC3-II/LC3-I in HSOS mice, but it alleviated the downregulation of Atg12, albeit not significantly. Thus, these results showed that bicyclol pretreatment ameliorates the abnormality in *CYP450* isoforms.

4 | DISCUSSION

The ingestion of *Gynura segetum* was to self-treat trauma, arthritis, pain, and hemorrhage in patients in China, which might lead to the incidence of HSOS despite low-dose consumption (the minimal ingestion was only 10 g).[5] Patients with HSOS induced by *Gynura segetum* may suffer from acute failure and subsequent life-threatening events if they do not receive timely treatment.[17] The results of the present study revealed that *Gynura segetum* (30 g/kg) administration for 4 weeks induced HSOS in mice, as shown by the increase in serum AST levels, the decrease in serum lipids, and the liver pathological changes, including central venous subendothelial damage and hemorrhage, severe sinusoidal congestion, and fibrosis in some portal areas, which are consistent with previous studies.[14,18] Bicyclol pretreatment (200 mg/kg) exerted an overall protective effect on HSOS induced by *Gynura segetum* in the current study, as evident by inhibiting the elevated serum ALT, raising the levels of serum lipids, and alleviating pathological changes caused by HSOS in mice.

CYP450 isozymes are widely involved in the metabolic activities of endogenous and exogenous substances, such as drugs and environmental compounds.[19] *CYP2a5* metabolizes carcinogens, such as nitrosamines and aflatoxin B1, into genotoxic metabolites and is involved in hepatocarcinogenesis, while toxic products of metabolism can induce liver damage.[20] *CYP2b10* and *CYP3a11* are the markers of two major members of the nuclear receptor superfamily, termed constitutional androstane receptor (CAR) and pregnane X receptor (PXR), respectively,[21] indicating that their levels are representative of the expressions of CAR and PXR. Furthermore, CAR and PXR enhanced the clearance of toxic by-products of endogenous and exogenous chemicals.[22] *CYP2c29* and *CYP2c37* belong to the *CYP2c*

subfamily that metabolizes arachidonic acid to bioactive eicosanoids.[23,24] *CYP7b1* mediates several physiological functions, mainly in the liver as bile salt synthesis.[25] In addition, a few drugs and poisons are known to alter the activity of *CYP450* isozymes, such as isoniazid, rifampin, and carbon tetrachloride.[26,27] Some therapeutic drugs can exert therapeutic effects by altering the activity of *CYP450* such as glycyrrhizin.[28] Several previous studies have documented that pyrrolizidine alkaloids are metabolized to dihexyl phthalate esters by hepatic *CYP450* isozymes (*CYP2B* and *CYP3A*) after absorption in the intestine[29–31]; however, whether HSOS induced by *Gynura segetum* poisoning affects the expressions of *CYP450* isozymes is yet to be elucidated. *CYP450* levels might affect various signal transduction pathways that alter the cell cycle, causing apoptosis or aberrant cell growth, and thereby tumorigenesis.[32] Therefore, the mRNA expressions of several *CYP450* isozymes, including *CYP2a5*, *CYP2b10*, *CYP2c29*, *CYP2c37*, *CYP3a11*, and *CYP7b1*, were measured in the present study. The results revealed that *CYP2a5* mRNA expression was notably upregulated in HSOS induced by *Gynura segetum* compared to the control group, while the mRNA expressions of *CYP2b10*, *CYP2c29*, *CYP2c37*, *CYP3a11*, and *CYP7b1* were significantly downregulated. However, bicyclol pretreatment ameliorates the abnormalities of specific *CYP450* isoforms at the mRNA levels during HSOS; the bidirectional regulation might contribute to the hepatoprotective effect. Similarly, Yao et al.[9] reported that bicyclol significantly attenuated the reduction in *CYP2C6*, *CYP3A1/2*, and *CYP2C11* activity and mRNA expression in partial hepatectomy (PH) rat. Bicyclol can upregulate the mRNA and protein expressions of *CYP3A1* and *CYP2E1*. These results showed that bicyclol pretreatment ameliorates abnormality in *CYP450* isoforms during liver regeneration after PH.

Apoptosis is programmed cell death that occurs in a physiological process and in numerous pathological processes.[33] Several drugs or chemicals may cause direct and predictable liver injury, accompanied by hepatocyte apoptosis, which is also involved in *Gynura segetum*.[14,33–37] Consistent results in the current study showed that *Gynura segetum* significantly increases TUNEL-positive cells and upregulates the expression of apoptosis-executing protein Caspase-3 compared to the control. Interestingly, the expression of apoptosis suppressor protein Bcl-2 was dramatically upregulated. Previous studies have shown that bicyclol acts as a hepatoprotective agent by anti-apoptosis in liver injury mice.[38–40] In HepG2 cells intoxicated with D-GalN in vitro, pretreatment with bicyclol remarkably attenuated apoptosis, and the underlying mechanism includes suppression of caspase-3 activity.[41] However, in the present study, an inconspicuous decrease in TUNEL-positive cells and Caspase-3 protein expression after bicyclol pretreatment suggested that apoptosis level was not inhibited. Conversely, apoptosis inhibitory protein Bcl-2 was reduced by bicyclol pretreatment, which could be attributed to the protection of liver damage as the damage was milder than that in the HSOS group. Therefore, bicyclol pretreatment has a potential protective effect owing to the downregulation of inhibitory protein Bcl-2 in HSOS mice.

Autophagy plays a role in maintaining the physiological liver cellular and metabolic homeostasis, while malfunction or dysregulation of autophagy is linked to diverse liver diseases, such as non-alcoholic fatty liver disease, viral hepatitis, drug-induced liver injury, and hepatocellular carcinoma.[42,43] Consequently, a novel therapeutic approach for targeting autophagy is emerging for liver diseases.[44] Our previous study recently reported that autophagy was impaired in response to *Gynura segetum*-induced HSOS in mice.[14] The finding was further extended by the present results showing that the levels of Atg12 and LC3-II/LC3-I proteins were markedly downregulated in HSOS mice, while that of Bcl-2, a blocker of autophagy occurrence, was dramatically upregulated.[45] Bicyclol pretreatment substantially reversed the altered protein expressions of both LC3-II/LC3-I and Bcl-2, while that of Atg12 was less pronounced. These results suggested that bicyclol contributes to the hepatoprotective effect in HSOS mice via recovery of autophagy. Compared to other hepatoprotective and anticoagulant drugs, bicyclol can exert both hepatoprotective and enzyme-lowering effects and also improve the abnormalities of CYP450 induced by *Gynura segetum* and alleviate the downregulation of autophagy of hepatocytes, thereby providing a new strategy therapeutic for HSOS.

5 | CONCLUSION

In conclusion, the present study demonstrated that bicyclol effectively attenuated HSOS induced by *Gynura segetum*, which could be attributed to the regulated expressions of CYP450 isozymes and alleviated the downregulation of autophagy.

As bicyclol exerts these anti-HSOS effects, it may be further assessed for the treatment of HSOS induced by *Gynura segetum*.

AUTHOR CONTRIBUTIONS

Design of the study: LH, YXM, and YJZ; Preparation of Figures and Tables, Interpretation of the data and Writing the original draft: YJZ, WJY, and JS; Technical procedures: YJZ, WJY, JS, SJP, ZX, and XZP; Performing statistical analysis: ZH and ZX; Revision and modification of the manuscript: LH and YXM.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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