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ORIGINAL RESEARCH

Protective Effect of Hordenine on Concanavalin A-Induced Hepatic Injury

Yanzhan Liu[1,*], Yu Wang[2,*], Mingchao Jiang[1,*], Qilian Zhang[3], Mingtao Yang[1], Fang Sun[1], Zhengguang Xu[1], Tao Liu[4], Jing Yao[1,5]

[1]School of Basic Medicine, Jining Medical University, Jining, 272067, People's Republic of China; [2]Department of Pharmacy, Xuzhou City Hospital of TCM, Xuzhou, 221000, People's Republic of China; [3]Department of Pathology, People's Hospital Affiliated to Shandong First Medical University, Jinan, 271100, People's Republic of China; [4]Affiliated Hospital of Jining Medical University, Department of Otolaryngology, Head and Neck Surgery, Jining, 272067, People's Republic of China; [5]Jining Key Laboratory of Pharmacology, Jining Medical University, Jining, 272067, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jing Yao, School of Basic Medicine, Jining Medical University, 133 Hehua Road, Jining, 272067, People's Republic of China, Tel/Fax +863616272, Email yjing_87@163.com

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Objective: This study aimed to investigate the protective effects of hordenine on concanavalin A (ConA)-induced immune liver injury (ILI).
Methods: Liver coefficients and spleen coefficients were determined. The serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) were detected. Hematoxylin-eosin (H&E) staining was used to observe the pathological changes of the liver, spleen, and thymus. Immunohistochemical experiments were performed to detect the expression of inflammatory factors interleukin (IL)-6, IL-1β, and tumor necrosis factor alpha (TNF-α) in liver tissues. Superoxide dismutase (SOD) and malondialdehyde (MDA) contents were detected. TdT-Mediated dUTP Nick-End Labeling (TUNEL) staining was used to observe the apoptosis of liver cells. Western Blot was used to detect the expression of B-cell lymphoma-2 (Bcl-2), Caspase-3, Bcl-2-Associated X (Bax), kelch-like ECH-associated protein 1 (Keap1), nuclear factor erythroid 2–related factor 2 (Nrf2), and heme oxygenase-1 (HO-1).
Results: We found that hordenine alleviated liver and spleen enlargement, ameliorated pathological damage in the liver, spleen, and thymus, and reduced transaminase and inflammatory factor levels in the liver. Moreover, hordenine increased the level of SOD while decreasing the level of MDA, attenuated hepatic apoptosis, decreased the expression of caspase-3, Bax, and Keap1 and increased the expression of Bcl-2, Nrf2, and HO-1.
Conclusion: These results suggest that hordenine has a protective effect against ConA-induced immune liver injury, which may be related to its modulation of the Keap1/Nrf2/HO-1 pathway and inhibition of oxidative stress and apoptosis.
Keywords: hordenine, immune liver injury, oxidative stress, apoptosis, keap1/Nrf2/HO-1 pathway

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Introduction

Immune liver injury (ILI) is a progressive pathological condition mediated by the immune system, and its main pathological change is a large number of inflammatory cell infiltrations in liver tissues, along with a significant increase in the levels of aminotransferases and inflammatory factors, which is one of the most important factors leading to liver fibrosis, cirrhosis, and even hepatocellular carcinoma.[1–3] In recent years, the incidence of immune liver injury developing into severe liver failure or even death has increased significantly worldwide.[4] Currently, it is mainly treated with non-specific inhibitors or liver transplantation, but the adverse effects are numerous and expensive.[5,6] Therefore, it is urgent to explore the application of natural and effective drugs with low side effects for the treatment of immune liver injury.

Oxidative stress represents a pivotal mechanism in the progression of immune-mediated liver injury.[7] The nuclear factor erythroid 2-related factor 2 (Nrf2) serves as a pivotal regulatory component within the body's antioxidant stress system, playing a crucial role in maintaining homeostasis and cellular resilience.[8] The Kelch-like ECH-associated protein 1 (Keap1) is a negative regulatory factor of Nrf2.[9,10] Keap1/Nrf2/heme oxygenase-1 (HO-1) signaling is considered one of the most critical endogenous anti-oxidative stress pathways and is an important target in inflammation-related

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diseases.[11] After being attacked by reactive oxygen species (ROS), Nrf2 dissociates from Keap1 and subsequently binds to the antioxidant response element (ARE), further regulating the activities of target genes such as superoxide dismutase (SOD), catalase (CAT), and Phase II detoxifying enzymes in order to scavenge harmful substances such as ROS.[12] Apoptosis constitutes a significant aspect of programmed cell death and holds a crucial role in Concanavalin A (ConA)-induced immune liver injury. Both inflammation and oxidative stress can trigger apoptosis, the latter by activating the expression of various proteins that ultimately lead to mitochondrial dysfunction.[13,14]

Hordenine, chemically known as 4-(2-dimethylaminoethyl), belongs to the class of phenylethylamine alkaloids. It is derived from germinated barley and is prevalent in various plants, including grains, algae, cacti, as well as bitter oranges.[15] Hordenine exhibits a broad spectrum of bioactivities, specifically anti-inflammatory, antioxidant, antimicrobial, and antitumor properties, as documented in numerous studies.[16–18] Recent research has further revealed its potential to mitigate DSS-induced injury in mice with ulcerative colitis by reducing the expression of pro-inflammatory cytokines and modulating the S1P/S1PR1/STAT3 signaling pathway.[19] Hordenine has the ability to reduce neuroinflammation and exert neuroprotective effects by inhibiting the NF-κB and MAPK signaling pathways.[20] Studies have shown that hordenine can effectively enhance the activity of primary mouse hair papilla cells and accelerate hair regeneration through activation of the Wnt/β-catenin signaling pathway.[21] Hordenine has emerged as a promising candidate as an inhibitor of PDK3, suggesting its potential utility in the therapeutic management of cancer and a myriad of other PDK3-associated diseases.[22] However, the protective effect of hordenine against ConA-induced immune liver injury, alongside the underlying mechanisms responsible for this effect, remains an uncharted territory, deserving further exploration. Accordingly, the present study aims to delve into the protective effects of hordenine in ILI and elucidate the underlying mechanisms involved. To accomplish this, we have adopted a ConA-induced mouse model of ILI, with the aspiration of forging novel clinical avenues for the treatment of this condition.

Materials and Methods

Animals

Male BALB/c mice (aged 35~40 days old and weighing 18~20 g) from Jinan Pengyue Experimental Animal Breeding Co. Ltd. (Jinan, China). The rearing chamber for the experimental mice was stringently maintained at a precisely controlled temperature and humidity, ensuring optimal conditions conducive to their physiological well-being. Furthermore, a consistent circadian rhythm was established and upheld, pivotal in sustaining the normalcy of their biological processes and experimental outcomes. All animal experiments were approved by the Institutional Animal Care and Use Committee of Jining Medical University (2023-DW-145) and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, 2011).

Reagents

Hordenine (high-performance liquid chromatography-grade ≥95%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The chemical formula for hordenine is given in Figure 1A. Prednisolone was purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). ConA was purchased from Sigma-Aldrich. Hematoxylin and eosin (H&E) were purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). The Universal SP Kit and 3,3′-diaminobenzidine (DAB) were purchased from ZSGB-BIO Co., Ltd. (Beijing, China). ALT, AST, SOD and MDA kits were purchased from Nanjing Jiancheng Technology Co., Ltd (Nanjing, China). The TdT-mediated dUTP Nick-End Labeling (TUNEL) staining kit was purchased from Biyuntian Biotechnology Co., Ltd (Shanghai, China). Primary antibodies against Keap1, Nrf2, HO-1, caspase-3, Bcl-2, and Bax were purchased from Proteintech Group (Wuhan, China). Antibodies against β-actin was obtained from Affinity Biosciences (Cincinnati, OH, USA). Anti-mouse and anti-rabbit secondary antibodies were obtained from eBioscience (San Diego, CA, USA). Enhanced chemiluminescent (ECL) substrate was purchased from Beijing Labgic Technology Co., Ltd. (Beijing, China).

Establishment of ConA-Induced Immune Liver Injury Model

Drawing upon the previous reports,[23–26] we constructed a mouse model that could simulate the pathophysiological characteristics of ILI using ConA. After a period of one week for acclimatization and standard feeding, the mice were

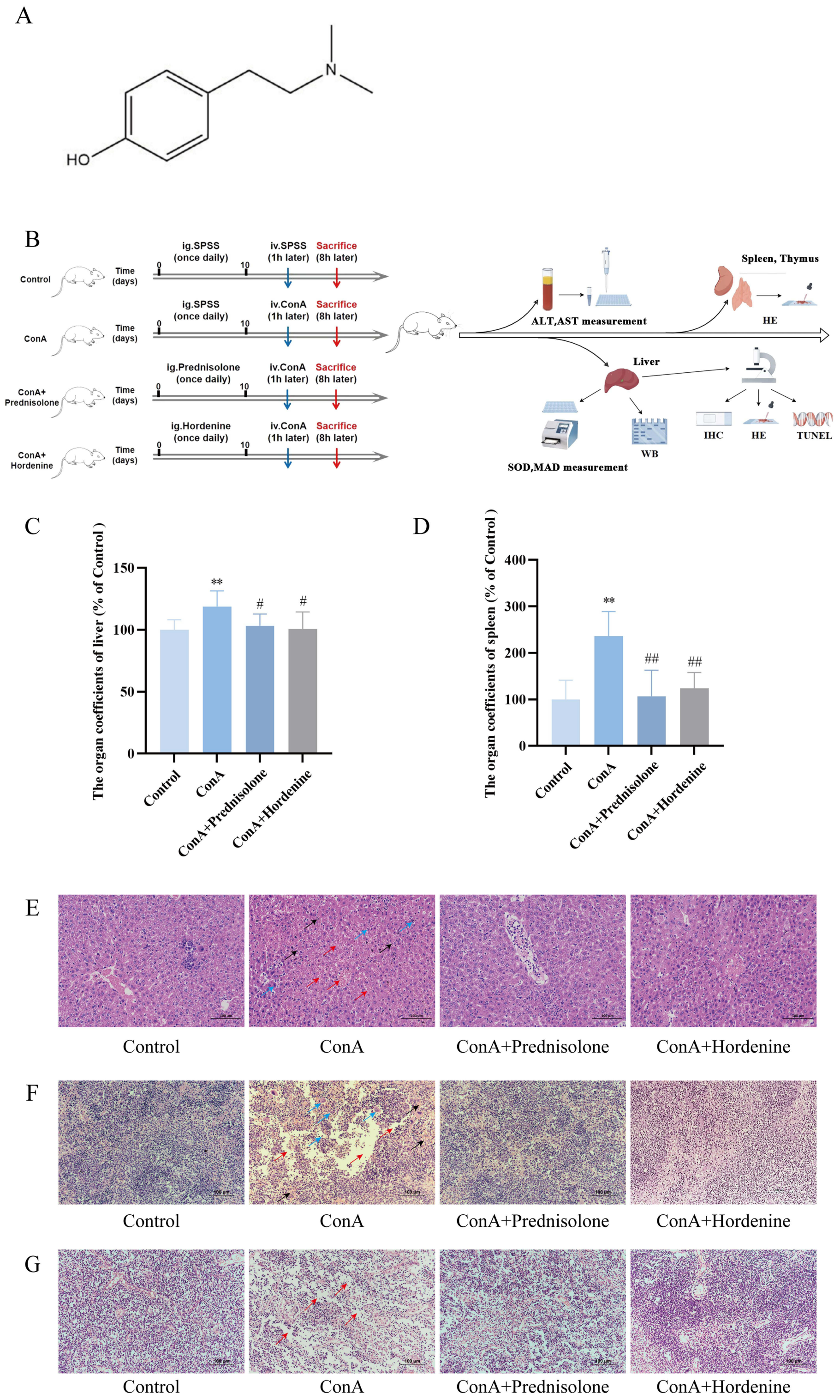

Figure 1 Hordenine alleviates ConA-induced the pathologic injury in mice. (**A**) The chemical structure of hordenine. (**B**) Animal model and experimental design. (**C**) Liver coefficient of each group of mice. (**D**) Spleen coefficient of each group of mice. ***p* < 0.01 compared with control group; #*p* < 0.05, ##*p* < 0.01 compared with ConA-treated group. Results were analyzed using one-way analysis of variance followed by Tukey's post-hoc test. The results are presented as mean±standard deviation, n=10 per group. (**E**) H&E staining histopathological images of the liver (magnification: ×200). The black arrow shows necrosis in the liver cells, and the red chuck shows congestion in the hepatic sinuses. The blue arrows show the infiltration of inflammatory cells, especially granulocytes. (**F**) H&E staining histopathological images of the spleen (magnification:×200). The red arrow indicates that a large number of cells in the splenic tissue have dissolved and disappeared, causing the tissue structure to become loose and disorganized. The black arrow indicates a rupture of this sinus, with diffuse bleeding in the tissue. The blue arrow shows the massive proliferation of macrophages. (**G**) H&E staining histopathological images of the thymus (magnification: ×200). The red arrow shows the dissolution and dissipation of a large number of cells in the thymus tissue, which becomes loosely structured.

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subsequently randomized into four distinct groups (n=10), each comprising ten individuals: control group, ConA-treated group, ConA+prednisolone (serving as a positive control) group, and ConA+hordenine group. The prednisolone group and the hordenine group were pre-treated with 10mg/kg prednisolone and 50 mg/kg hordenine via intragastric administration for 10 days. Both the control and ConA-treated groups underwent equal volumes of saline administration via gavage, ensuring a consistent and standardized treatment protocol. One hour after the last gavage, saline was injected into the tail vein of the control group, and the other groups established the ILI model by tail vein injection of ConA (20 mg/kg). Fresh blood samples were collected after 8 hours. Following the experimental protocol, the animals were humanely euthanized, and their vital organs (including the livers, spleens and thymuses) were collected. The liver, spleen and thymus tissues were fixed in 4% paraformaldehyde for H&E staining, and the remaining liver tissues were stored in liquid nitrogen for protein blot analysis.

The Weighing of Organs

The livers and spleens of the mice were washed in saline solution. Following this, the organs were subsequently placed on weighing paper. An electronic balance was then employed to measure the mass of these organs, which was utilized to calculate the liver and spleen indices. The formulas adopted for these calculations were as follows: liver index = (liver mass /body mass)×100%, and spleen index = (spleen mass /body mass)×100%.

The Detection of the Levels of Alanine Transaminase (ALT) and Aspartate Transaminase (AST)

To evaluate liver functionality in mice, we conducted a assessment by measuring the serum levels of ALT and AST. Following the collection of mouse blood samples, we promptly processed them through centrifugation to isolate the serum. Subsequently, we employed a spectrophotometric assay to determine the activity of ALT and AST within the serum.

H&E Staining

Liver, spleen, and thymus tissues were fixed in 4% paraformaldehyde for 24 hours, then dehydrated and embedded, sectioned at a thickness of 4 μm, and routinely stained with H&E staining to observe the histopathological changes of the liver, according to the previous reports.[27]

Immunohistochemical (IHC)

Liver tissue sections were deparaffinized, hydrated and blocked endogenous peroxidase activity by incubation with 30% hydrogen peroxide (H_2O_2), and then washed three times with phosphate buffer. The sections were incubated in 10% goat serum for 20 min at room temperature to block non-specific binding, and then incubated with interleukin (IL)-6, IL-1β, and tumor necrosis factor alpha (TNF-α) antibodies at 4°C overnight. The slices were washed three times with phosphate buffer and incubated with secondary antibodies for 30 minutes at room temperature. The sections were stained with DAB, restained with hematoxylin, dehydrated and dried, and then observed under a microscope and photographed.

The Detection of the Levels of SOD and MDA

The liver tissues were lysed and homogenized, and the supernatant was centrifuged at 4°C at 3000 rpm/min for 10 min. And the levels of SOD and MDA were tested according to the kit instructions.

TUNEL Assay

Liver tissue sections were procured to the colorimetric TUNEL apoptosis assay kit's instruction. Subsequently, they were sealed with neutral resins for preservation and were then observed under a microscope, with photographs being captured for documentation and analysis.

Western Blot

Upon obtaining frozen liver tissue, the proteins contained within were extracted utilizing RIPA buffer. Tissues were lysed and analyzed by Western blotting as previously described.[16] Blots were probed with anti-B-cell lymphoma-2 (Bcl-2), anti-caspase-3, anti-Bcl-2-associated X (Bax), anti-Keap1, anti-Nrf2 and HO-1 primary antibodies. β-actin was probed as an internal loading control.

Statistical Analysis

All results are presented as the mean ± standard deviation of triplicate experiments. The statistical was performed using GraphPad Prism software (San Diego, CA, United States). Independent sample *t*-tests and one-way ANOVA were conducted for comparisons between two groups and multiple groups, respectively. The *p*-values less than 0.05 were considered statistically significant.

Results

Hordenine Alleviates ConA-Induced the Pathologic Injury in Liver, Spleen and Thymus

All animal procedures and assays are shown in Figure 1B. In our investigation, we observed a notable elevation in the liver and spleen coefficients among mice exposed to the ConA group, in contrast to the control group. Intriguingly, the administration of hordenine exhibited a remarkable capacity to mitigate this ConA-mediated enlargement of both organs, as depicted in Figure 1C and D. This finding underscores the potential therapeutic role of hordenine in alleviating the pathological consequences associated with immune-mediated liver and spleen enlargement in ILI mice.

Upon histopathological image analysis, the liver tissues from mice in the normal control group exhibited normal morphology and structure of hepatocytes under light microscopy. Hepatocyte cords were arranged in a neat and orderly fashion, devoid of any inflammatory cell infiltration or signs of hepatocyte degeneration or necrosis. Conversely, the liver tissue of mice in the model group displayed profound pathological alterations, characterized by swollen hepatocytes with turbid cytoplasm, indistinct cell boundaries, and disrupted hepatic cords. Additionally, there was evident bruising accompanied by severe inflammatory cell infiltration, alongside numerous punctate necrotic areas within the liver parenchyma. These findings indicate the induction of severe liver injury following tail vein injection of ConA in these mice. In contrast, the cellular morphology of liver tissue in the hordenine-treated group demonstrated a tendency towards improvement, with a marked reduction in the number of inflammatory cells compared to the model group. Necrotic areas in the liver parenchyma were either diminished or absent, and bruising in hepatic sinusoids was alleviated, suggesting a protective effect of hordenine against ConA-induced liver damage (Figure 1E).

Furthermore, H&E histopathological staining revealed extensive cell lysis and structural loosening within the splenic tissue of mice in the model group. This disruption led to the rupture of blood sinuses, resulting in diffuse hemorrhage and pronounced macrophage proliferation (Figure 1F). Similarly, ConA caused substantial cell lysis and structural loosening in thymic tissue, further highlighting its deleterious effects on the immune system. And hordenine reduced the above pathological damage (Figure 1G).

In summary, these observations underscore the pathological changes elicited by ConA in liver, spleen, and thymus tissues, as well as the mitigating role of hordenine in ameliorating such damage.

Hordenine Reduces Serum Levels of ALT and AST in Con A-Induced Mice

To assess ConA-induced liver injury in mice, the serum levels of ALT and AST were detected. The results showed that ALT and AST levels were significantly higher in the ConA-treated group compared to the control group, which were dramatically decreased in hordenine-treated group (Figure 2).

Hordenine Reduces the Expression of Inflammatory Factors Induced by ConA in Mice

The IHC findings indicated a pronounced elevation in the expression levels of IL-6, IL-1β and TNF-α proteins in hepatic tissues subsequent to ConA injection. However, hordenine treatment was observed to markedly diminish the expression of these inflammatory factors, suggesting a potential therapeutic effect in mitigating the inflammatory response (Figure 3).

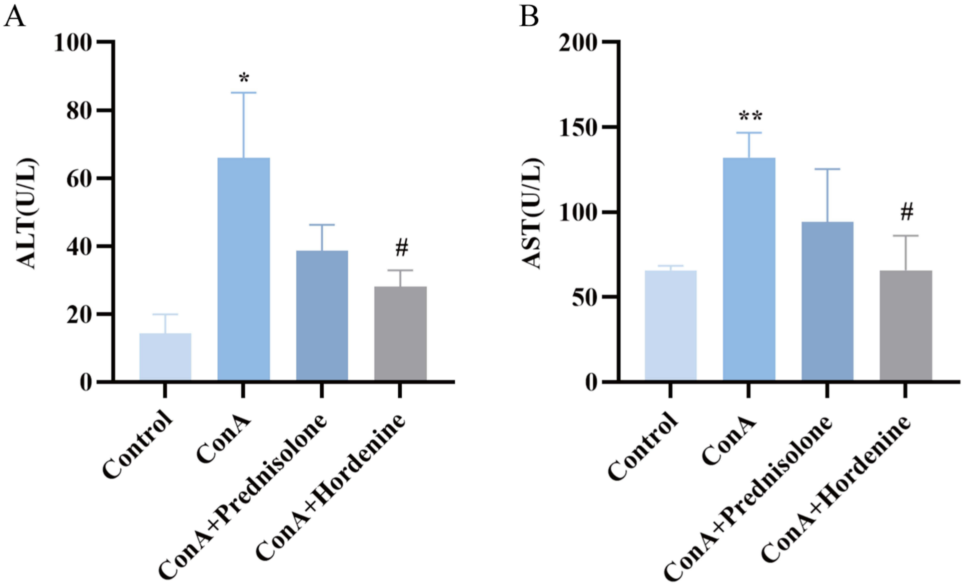

Figure 2 Hordenine reduces serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in Con A-induced mice. (**A** and **B**) Serum ALT and AST activities. *$p < 0.05$, **$p < 0.01$ compared with control group; # $p < 0.05$ compared with Con A-treated group. Results were analyzed using one-way analysis of variance followed by Tukey's post-hoc test. The results are presented as mean±standard deviation, n=3 per group.

Hordenine Attenuates Hepatic Apoptosis in a Model of ILI in Mice

TUNEL staining results showed that hordenine alleviated the apoptosis of hepatocytes in the liver tissues of ILI mice (Figure 4A and B) Meanwhile, Western blot results showed that hordenine significantly increased the expression of the anti-apoptotic protein Bcl-2 and decreased the expression of the pro-apoptotic proteins caspase-3 and Bax (Figure 4C–F).

Hordenine Inhibits ConA-Induced Oxidative Stress

The analysis results showed the level of SOD was significantly decreased, and the level of MDA was markedly increased in the ConA-treated mice. However, hordenine increased the level of SOD and decreased the level of MDA (Figure 5).

Hordenine Regulates the Expression of Keap1/Nrf2/HO-1 Pathway in Liver Tissues

To further explore the effect of hordenine on oxidative stress, we detected the expression of Keap1/Nrf2/HO-1 pathway in liver tissues. Western blot analysis revealed that Keap1 expression underwent a notable upregulation, while the expression of Nrf2 and its downstream effector HO-1 were significantly downregulated in liver tissues subsequent to ConA injection. In contrast, the administration of hordenine produced a remarkable reversal of this trend (Figure 6). This upregulation of Nrf2 and HO-1 suggests that hordenine exerts a potent protective effect against oxidative stress, possibly through the activation of the Nrf2-mediated antioxidant response.

Discussion

ConA, a phytagglutinin derived from Canavalia ensiformis,[28] exhibits a distinct capacity to interact with and activate Kupffer cells within the hepatic microenvironment.[24] This activation process is intricately mediated by hepatic sinusoidal endothelial cells, which serve as a pivotal intermediary. Upon engagement, ConA triggers a cascade of immunological events, including the potent stimulation of $CD4^+$ T cells. Consequently, these activated T cells and Kupffer cells collaboratively synthesize and secrete an array of inflammatory cytokines, notably including TNF-α, IL-1, IL-2, and IFN-γ. This robust inflammatory response further amplifies the activity of Kupffer cells, underscoring the profound impact of ConA on hepatic immunopathology.[29,30] Mediated by the secretion of cytokines and associated adhesion molecules by activated immune cells, ConA triggers an excessive immune response, an inflammatory cascade, and excess production of free radicals in the liver. In turn, these cytokines can trigger apoptosis and necrosis of liver cells, leading to liver dysfunction and damage. Therefore, the mouse model of ConA-induced liver injury has

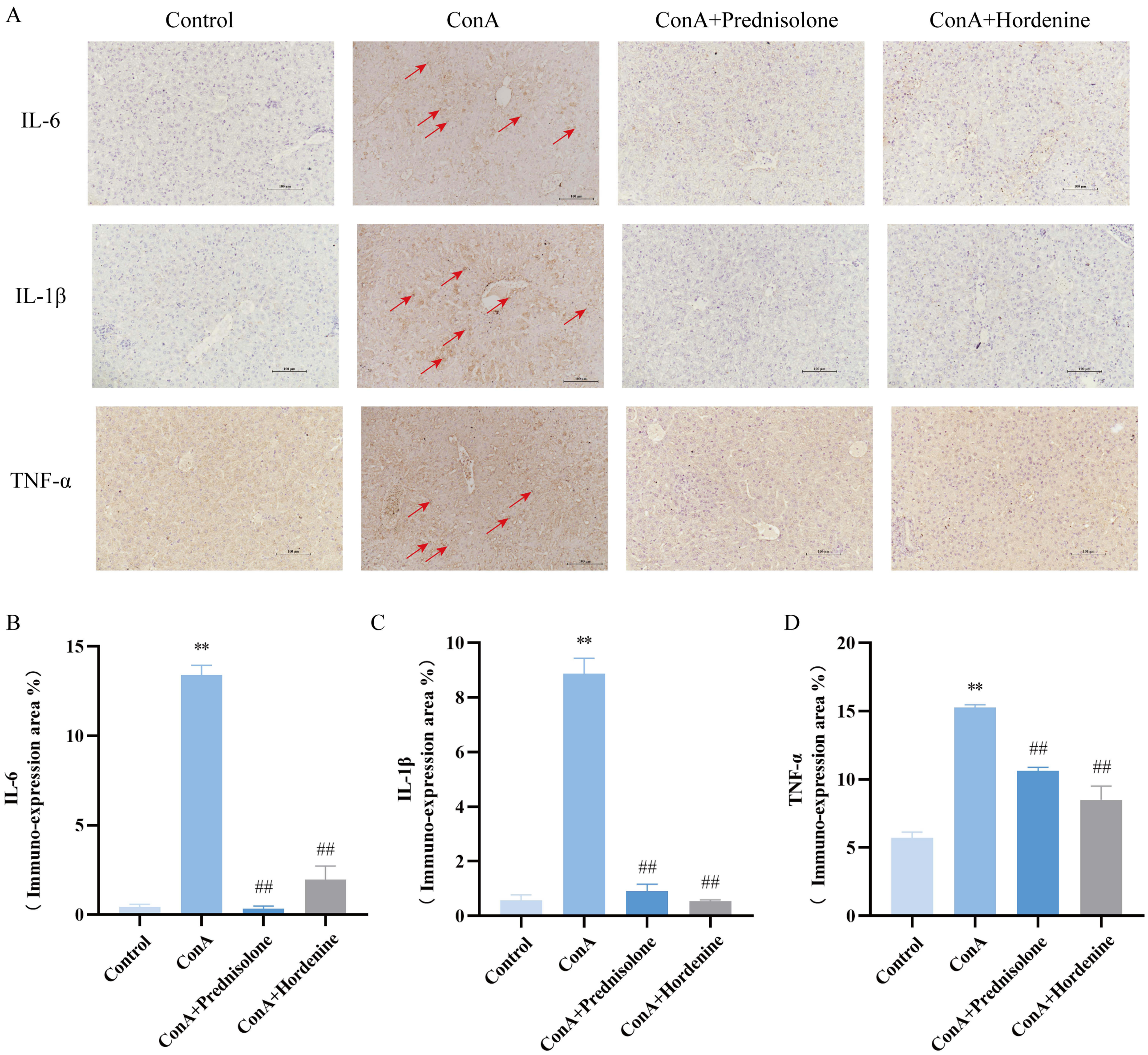

Figure 3 Hordenine reduces the expression of inflammatory factors induced by ConA in mice. (**A**) IHC staining images of IL-6, IL-1β and TNF-α in liver tissues (magnification: ×200). The red arrows indicate positive expression in liver tissues. (**B**–**D**) Results are presented as the mean ± standard deviation. $^{**}p < 0.01$ compared with control group, $^{\#\#}p < 0.01$ compared with Con A-treated group. Results were analyzed using one-way analysis of variance followed by Tukey's post-hoc test. The results are presented as mean±standard deviation, n=3 per group.

garnered extensive utilization in the scientific community, serving as a pivotal tool for the in-depth exploration of human immune-mediated liver injury diseases. In this study, we observed severe damage to the liver, spleen, and thymus in ConA-induced mice, with significant inflammatory cell infiltration in the liver, especially in the confluence area. In addition, the hepatic cord showed significant disorganization with extensive cell necrosis, underscoring the severity of the inflammatory damage. Compared with the control group, the level of ALT and AST in the ConA-treated group was significantly increased, which indicated that the model was successfully constructed. Notably, these pathological changes were reversed after administration of hordenine, indicating its potential therapeutic efficacy in alleviating the harmful effects of ConA-induced liver injury. This finding highlights the protective effect of hordenine against immune-mediated liver disease and its mechanisms.

Apoptosis, a highly orchestrated process of programmed cell death, serves as the main cause in liver injury. Among the pivotal factors contributing to this phenomenon, oxidative stress emerges as a significant player.[31,32] Elevated levels of ROS can compromise mitochondrial integrity by altering membrane permeability, thereby initiating a cascade of apoptotic events.

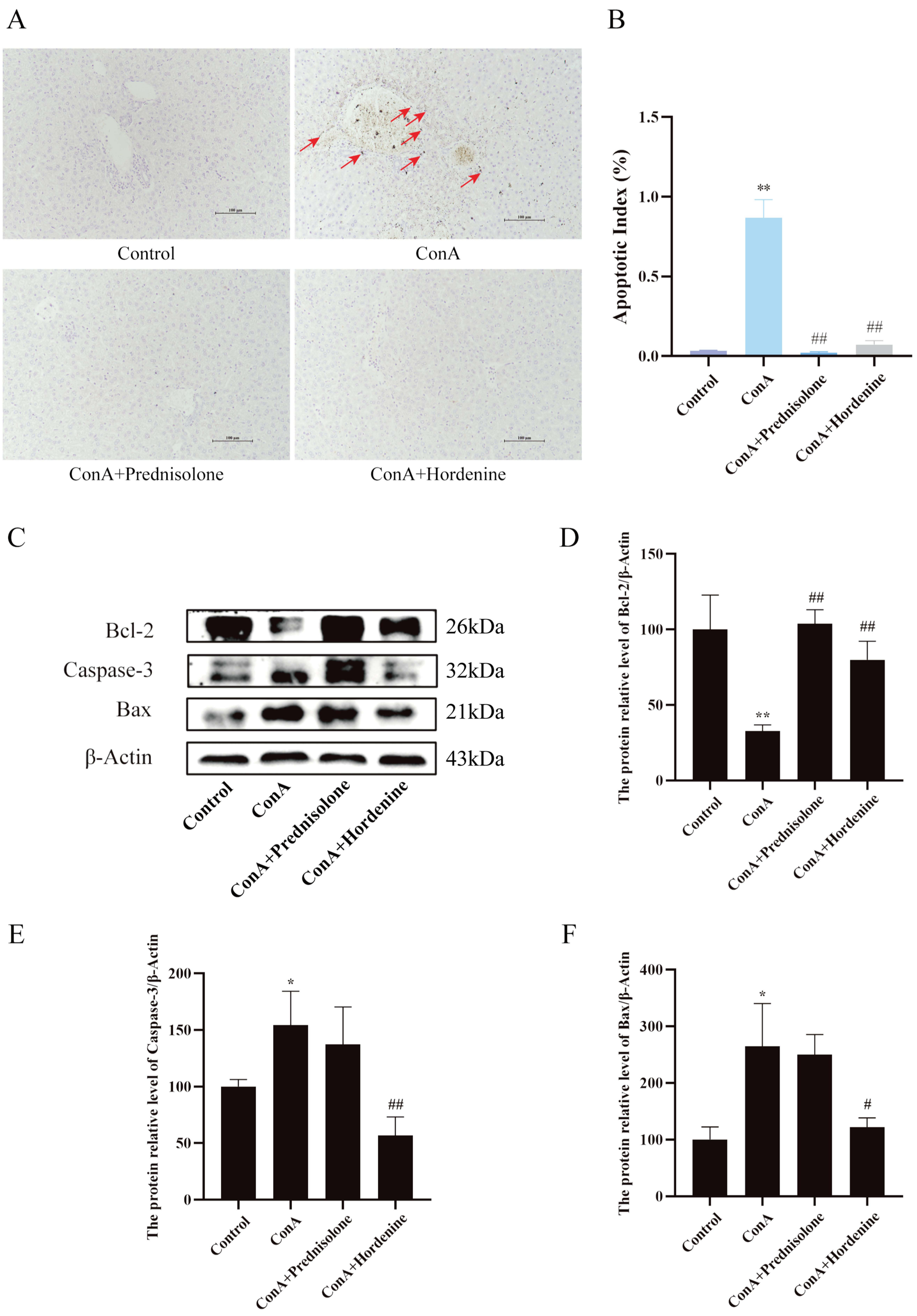

Figure 4 Effects of Hordenine on Hepatic Apoptosis in a Model of Immune-Induced Liver Injury. (**A**) Images of TUNEL staining of liver tissue. (**B**) Results are presented as the mean ± standard deviation. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with Con A-treated group. (**C–F**) Western blotting was performed to analyze the expression of Bcl-2, caspase-3 and Bax. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with Con A-treated group. Results were analyzed using one-way analysis of variance followed by Tukey's post-hoc test. The results are presented as mean±standard deviation, n=3 per group.

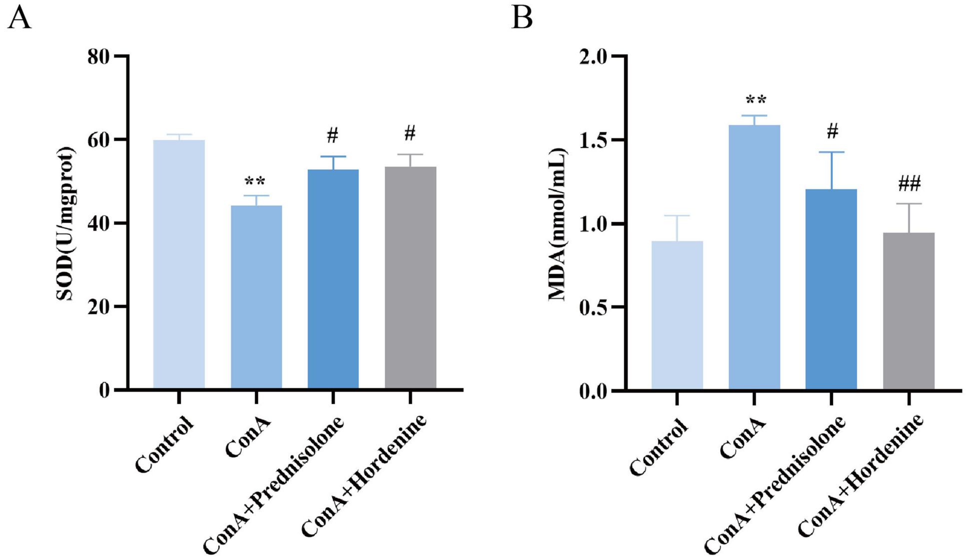

Figure 5 Effects of hordenine on the levels of SOD, MDA in liver tissues. (**A** and **B**) The levels of SOD, MDA in liver tissues. $^{**}p < 0.01$ compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with Con A-treated group. Results were analyzed using one-way analysis of variance followed by Tukey's post-hoc test. The results are presented as mean±standard deviation, n=3 per group.

This cascade leads to DNA fragmentation and ultimately accelerates the apoptotic process.[33,34] The intricate balance between life and death within mitochondria is tightly regulated by a repertoire of proteins, notably the pro-apoptotic Bax, Bid, and Bak, and their anti-apoptotic counterparts Bcl-2 and Bcl-xL.[35–37] Specifically, Bax promotes the liberation of cytochrome C, which in turn escalates ROS production, fostering excessive apoptosis in hepatocytes.[38] Furthermore, the intricate apoptotic machinery heavily relies on the caspase family of enzymes, which facilitate intracellular proteolysis and DNA degradation, culminating in apoptotic cell demise.[39] Notably, Caspase-3, a cornerstone member of this family, is colloquially referred to as the "death protein" due to its pivotal role in activating both the death receptor and mitochondrial apoptotic pathways.[40] Histopathological analysis via H&E staining revealed that ConA-induced liver injury was characterized by a conspicuous presence of necrotic regions interspersed with a substantial number of apoptotic cells. Therefore, we studied the apoptosis of liver tissue in depth. Our findings, as evidenced by TUNEL staining and Western blot analyses, indicated that hordenine exhibited a pronounced inhibitory effect on ConA-induced apoptosis. Specifically, it attenuates the upregulation of Caspase-3 and Bax while augmenting the expression of Bcl-2. These observations suggested that hordenine effectively modulates the apoptotic cascade, mitigating abnormal liver cell death and thereby alleviating immune-mediated liver damage in mice.

In the context of liver diseases, oxidative stress induced by oxygen free radicals constitutes a ubiquitous pathophysiological cornerstone.[41] This phenomenon is particularly evident in immune-mediated liver injury, where a disrupted redox equilibrium within the liver gives rise to profound oxidative stress. This condition, in turn, unleashes an unchecked inflammatory response, which acts as a catalyst, further intensifying oxidative stress. The reciprocal amplification forms a cycle, relentlessly exacerbating liver injury and underscoring the intricate interplay between oxidative stress and inflammation in the pathogenesis of this condition.[42] SOD activity and MDA content reflect the ability of scavenging free radicals and the degree of lipid peroxidation damage.[32] In the present study, ConA administration increased the levels of lipid peroxidation products, which have been shown to be associated with oxidative stress. Hordenine significantly decreased the MDA content and increased the SOD content compared to the ConA group.

The Keap1/Nrf2/HO-1 pathway plays a pivotal role in mitigating oxidative stress-induced damage across a broad spectrum of tissues and organs, including the heart, brain, liver, lungs, and the intricate nervous system.[43–48] HO-1, a pivotal member of the phase II detoxifying enzyme family, elicits a downstream signaling cascade that exerts protective mechanisms against oxidative stress across various organs[49] (Figure 7). Furthermore, its pivotal role extends to catalyzing the degradation of heme, thereby effectively dampening inflammatory responses orchestrated by neutrophils, macrophages, and lymphocytes.[50] This multifaceted functionality underscores the crucial importance of HO-1 in maintaining homeostasis and mitigating the deleterious effects of oxidative stress and inflammation. Western blot results

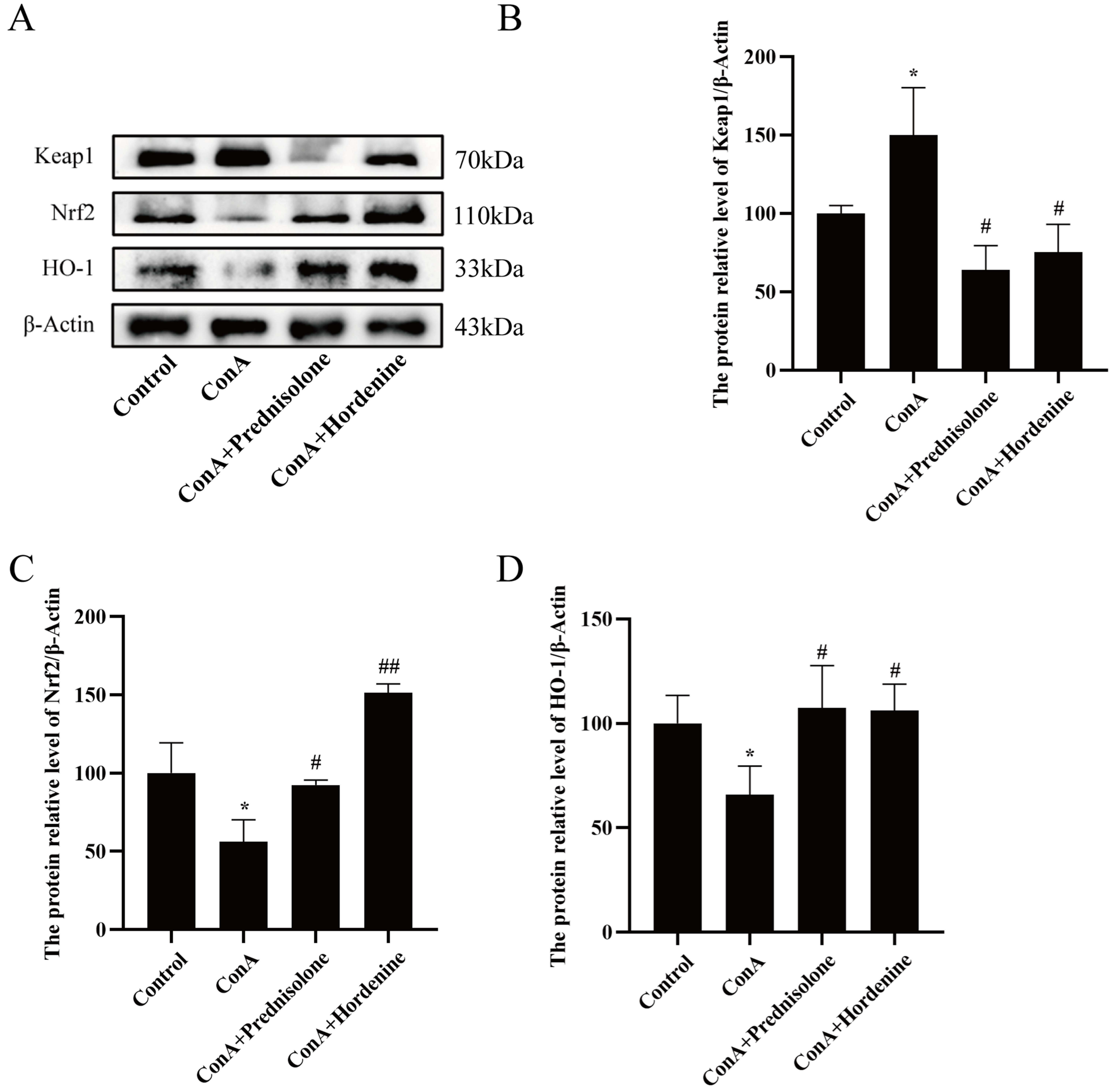

Figure 6 Effects of hordenine on the expression of the Keap1/Nrf2/HO-1 signal pathway in mice treated with Con A. (**A**–**D**) Western blotting analysis of Keap1, Nrf2 and HO-1 in liver tissue extracts. *$p < 0.05$ compared with control group; #$p < 0.05$, ##$p < 0.01$ compared with Con A-treated group. Results were analyzed using one-way analysis of variance followed by Tukey's post-hoc test. The results are presented as mean±standard deviation, n=3 per group.

indicated that hordenine exerted a protective effect against ILI by modulating the Keap1/Nrf2/HO-1 pathway and thereby inhibiting oxidative stress. Nonetheless, the intricate mechanisms underlying the therapeutic efficacy of hordenine in treating liver injury remain an area of ongoing investigation, necessitating further studies to elucidate its precise mode of action. Although we focused on Keap1/Nrf2/HO-1 pathways, other potential mechanisms, such as TLR4/MyD88/NF-κB signaling (given hordenine's anti-inflammatory properties), autophagy modulation (linked to liver injury recovery), and mitochondrial function regulation, remain to be explored.

Although the current study demonstrates the acute hepatoprotective effects of hordenine, its long-term benefits, particularly in the prevention of fibrosis, require further investigation through extended-duration studies, which are currently being designed. The immediate next step is to conduct extended-duration studies in carbon tetrachloride-induced fibrosis models to evaluate hordenine's long-term efficacy.

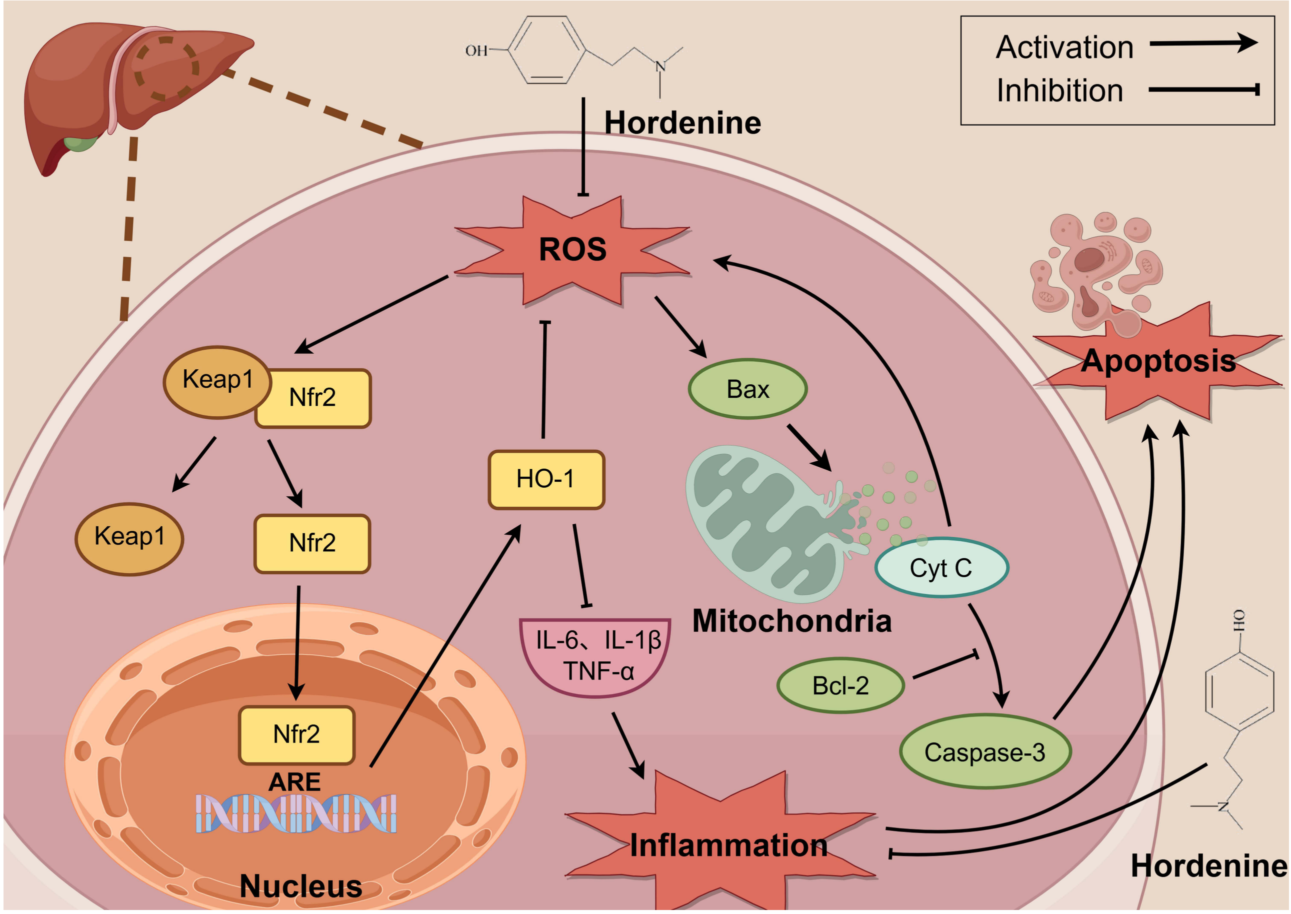

Figure 7 The role of the kelch-like ECH-associated protein 1 (Keap1)/nuclear factor erythroid 2–related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) and Bcl-2-Associated X (Bax)/B-cell lymphoma-2 (Bcl-2)/Caspase-3 signaling pathways in hordenine's inhibition of Concanavalin A -induced immune liver injury.

Conclusion

This study underscores the remarkable potential of hordenine as a therapeutic agent in addressing ConA-induced immune liver injury. Hordenine significantly ameliorates liver, spleen, and thymus damage, concurrently suppressing the expression of inflammatory factors and alleviating oxidative stress through the modulation of the Keap1/Nrf2/HO-1 pathway. Notably, its ability to inhibit hepatic apoptosis underscores its pivotal role in preserving liver function. These findings collectively suggested that hordenine represented a promising candidate for the effective treatment of immune-mediated liver injury, offering a novel therapeutic avenue for future clinical exploration.

Data Sharing Statement

Data will be made available on request.

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.

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Disclosure

The authors declare no conflict of interest.

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