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Swertiamarin or heat-transformed products alleviated APAP-induced hepatotoxicity via modulation of apoptotic and Nrf-2/NF-κB pathways

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ABSTRACT

Objective: Swertiamarin (STM) belongs to iridoid class of compounds, and the heat-transformed products (HTPS) are produced by STM in the process of drug processing. The purpose of this study was to explore the protective effect and mechanism of STM or HTPS on acetaminophen (APAP)-induced hepatotoxicity.

Methods: Mice and L-O2 cells were given APAP to establish the hepatotoxicity model *in vivo* and *in vitro*. The effects of STM or HTPS on oxidative stress, inflammation, and apoptosis induced by APAP were evaluated, with N-acetylcysteine (NAC) as a positive control.

Results: STM or HTPS reduced the APAP-induced apoptosis of L-O2 cells and significantly alleviated the liver injury index induced by APAP ($p < 0.01$, 0.005) Interestingly, HTPS had better protective effect against APAP-induced hepatotoxicity than STM ($p < 0.05$). In addition STM or HTPS improved the histological abnormalities; inhibited lipid peroxidation and reduced the level of inflammatory mediators. They also activated the defense system of nuclear factor erythroid 2 related factor 2 (Nrf-2) and inhibited nuclear factor-κ B (NF-κB).

1. Introduction

Liver plays a key role in the human body. It is well known that viruses, drugs, alcohol and toxic chemicals can lead to liver injury [1]. APAP is one of the most widely used analgesic and antipyretic drugs, and toxic metabolites can be neutralized by binding to endogenous GSH at the recommended dose of APAP. When high-dose of APAP is administered, a large amount of N-acetyl-*p*-benzoquinone imine (NAPQI) is formed which depletes GSH, leading to severe oxidative stress, hepatocyte necrosis and further producing

Abbreviations: APAP, Acetaminophen; STM, Swertiamarin; HTPS, Heat-transformed products; QYD, QingYeDan; NAC, N-acetylcysteine; GSH, Glutathione; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; MDA, Malondialdehyde; SOD, Superoxide dismutase; LDH, Lactate dehydrogenase; NO, Nitric oxide; IL-1β, Interleukin-1β; TNF-α, Tumor necrosis factor-α; IL-6, Interleukin-6; NF-κB, Nuclear factor kappa B; HO-1, Hemeoxygenase-1; Nrf-2, Nuclear factor erythroid 2-related factor 2.

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downstream inflammatory mediators and pro-inflammatory cytokines aggravating the injury [2–4]. Hepatotoxicity caused by APAP overdose leads to drug-induced acute liver failure in many developed countries [5]. N-acetylcysteine (NAC) is currently an effective drug for the treatment of APAP-induced hepatotoxicity, but its clinical application is limited due to the narrow therapeutic window and more side effects [6]. Therefore, it is necessary to find new drugs to achieve better safety and efficacy.

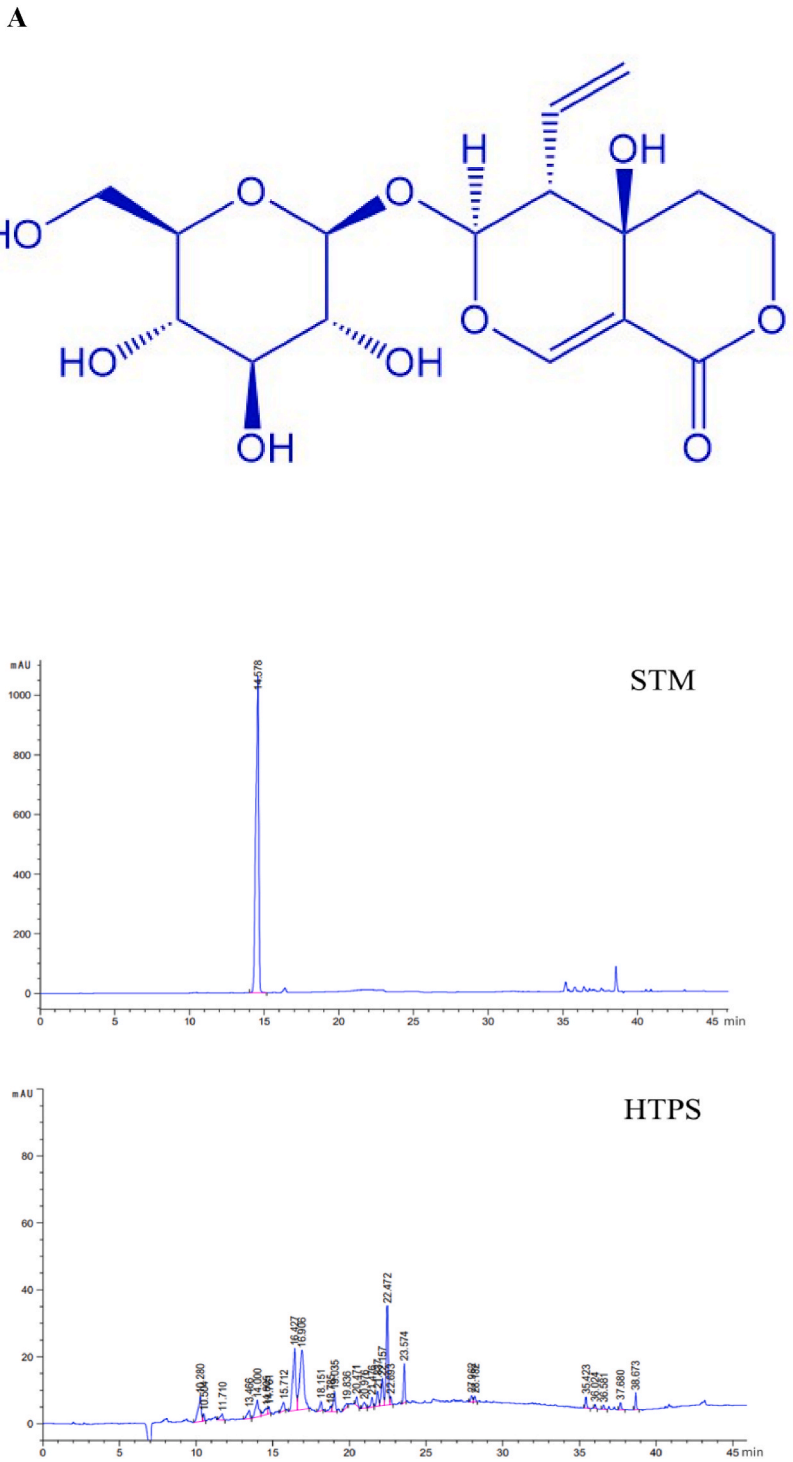

Fig. 1. Chemical structure of STM (A), HPLC chromatogram profiles of STM and HTPS (B), Method: column: octadecyl silane-bonded silica gel; eluent A: water; eluent B: methanol; gradient: 0–45 min, 95% A – 0% A, flow: 1 mL/min, absorption wavelength: 237 nm; column temperature: 30 °C.

STM (Fig. 1A), the main active ingredient of QYD tablets for the treatment of hepatitis, is recorded in the China Pharmacopoeia. It is derived from *Swertia mileensis* (Gentianaceae) and has significant anti-inflammatory, anti-oxidant and liver-protecting effects [7–9]. It has long been recognized by the Yi people for treating liver disorders, whether STM could alleviate APAP-induced hepatotoxicity remains unclear. Interestingly, previous studies showed that STM was converted into HTPS (Fig. 1B) during the production of drugs (QYD tablets) [10]. HTPS has potential effect on the liver injury caused by CCl_4 compared with STM, which has been reported in our previous study [11]. In current study, the protective effect and mechanism of STM and HTPS on APAP-induced hepatotoxicity are evaluated through *in vitro* and *in vivo* experiments.

2. Material and methods

2.1. Chemicals and reagents

STM ($C_{16}H_{22}O_{10}$, MW：374.37, purity: greater than 98%) was purchased from Push Bio-Technology (Chengdu, China) . APAP was obtained from Chinese Academy of Food and Drug Identification (Beijing, China). NAC was purchased from Macklin (Shanghai, China). Kits for the measurement of ALT, AST, GSH, MDA, SOD, LDH, and NO were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The ELISA kits for the determination of IL-1β, IL-6, and TNF-α were purchased from Link Bio. RPMI-1640 cell culture medium and fetal bovine serum were purchased from Gibco (USA). CCK-8 kits were purchased from Absin (Shanghai, China). V-FITC/PI staining kits were purchased from Procell (Wuhan, China). The primer anti-bodies (anti-Nrf-2, anti–HO–1, anti-Phospho–NF–κB-P65, anti-Phospho-Iκ Ba were purchased from American Proteintech. All the other chemicals and reagents were of analytical grade.

2.2. In vitro experiments

2.2.1. Cell culture

L-O2 cells were obtained from Cell Bank, Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The cells were cultured in RPMI-1640 supplemented with 10% [v/v] fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin, at 37 °C, in a humidified atmosphere of 5% CO_2.

2.2.2. Treatment of cells

L-O2 cells were plated at a density of 5×10^4 cells into 96/6-well plates per well and treated with 10 mM of APAP and different concentrations of STM or HTPS for 24 h. The administration groups were (n = 5): (1) NC: not treated control group; (2) APAP: APAP (10 mM); (3) NAC + APAP: NAC (250 μM) + APAP (10 mM); (4) LSTM + APAP: STM (25 μg/mL) + APAP (10 mM); (5) MSTM + APAP: STM (50 μg/mL) + APAP (10 mM); (6) HSTM + APAP: STM (100 μg/mL) + APAP (10 mM); (7) LHTPS + APAP: HTPS (25 μg/mL) + APAP (10 mM); (8) MHTPS + APAP: HTPS (50 μg/mL) + APAP (10 mM); (9) HHTPS + APAP: HTPS (100 μg/mL) + APAP (10 mM).

2.2.3. Measurement of cytotoxicity in APAP-induced L-O2 cells

APAP-induced cell damage is accompanied by inflammation and oxidative stress, and even leads to cell necrosis. L-O2 cells were treated according the above (2.2.2) , and APAP-induced cytotoxicity was measured by the CCK-8 method. Cell apoptosis was measured by flow cytometry using annexin V-FITC Apoptosis Detection Kit according to the manufacturer's instructions.

2.2.4. Measurement of ALT, AST, and LDH levels in APAP-induced L-O2 cells

ALT, AST, and LDH hepatic enzymes are released when hepatocytes are damaged [12]. The L-O2 cells were treated with a previously (2.2.2) described method and cell supernatants were collected for the analysis of ALT, AST, and LDH according to the instructions of the reagent manufacturer.

2.2.5. Measurement of MDA and SOD activity in APAP-induced L-O2 cells

Oxidative stress is one of the main causes responsible for APAP-induced hepatotoxicity [13]. L-O2 cells cultured in 6-well plates were treated with a previously (2.2.2) described method. Cells were detached using a rubber scraper and collected by centrifugation at 1000 rpm for 10 min at 4 °C. Cell pellets were sonicated in cold assay buffer. Cell lysates were centrifuged at 3000 rpm for 15 min at 4 °C. Whole homogenates were collected for assay. MDA and SOD in supernatants were detected according to the manufacturer's instructions. The level of oxidative stress was evaluated.

2.2.6. Measurement of IL-6 and TNF-α levels in APAP-induced L-O2 cells

APAP overdose trigger the activation of pro-inflammatory factors [14,15]. The L-O2 cells were treated with a previously (2.2.2) described method, the levels of IL-6 and TNF-α were detected in L-O2 cells induced by APAP, according to the manufacturer's instructions at absorbance 450 nm and reference 630 nm.

2.3. In vivo experiments

2.3.1. Animals and experimental groups

Male KM mice, aged 6–8 weeks, weighing 18–22 g, were purchased from Beijing Subefu Biotechnology Technology Co., Ltd. All

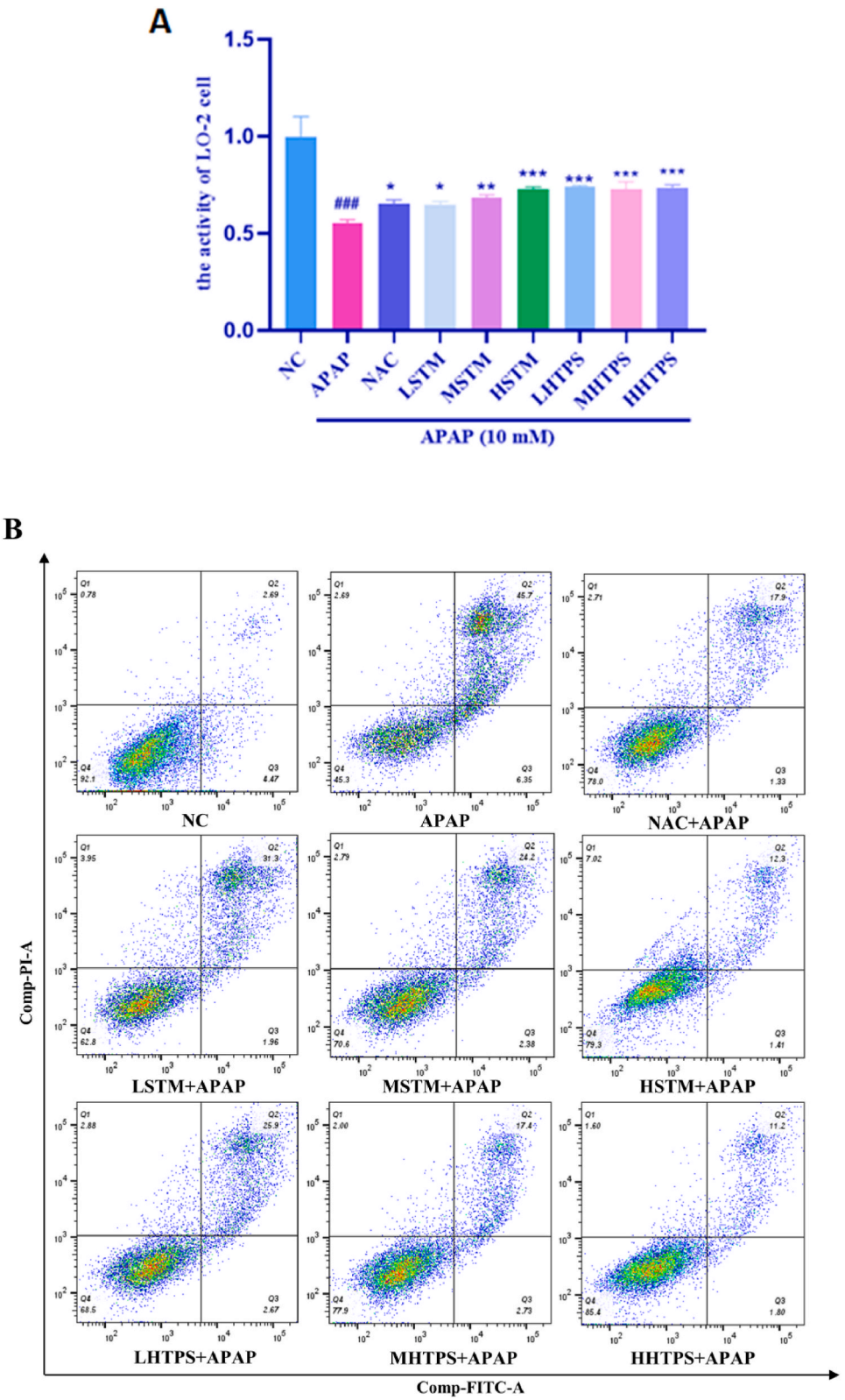

Fig. 2. STM or HTPS alleviated APAP-induced apoptosis in L-O2 cells. The activity of L-O2 cells was assayed by CCK-8 kit (3A). Cell apoptosis was detected using flow cytometry (3B). The values are given as the mean ± SD (n = 5). $^{###}p < 0.005$ versus NC group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ versus APAP group.

laboratory animals were carried out according to the ethical principles adopted in the Laboratory Animal Care and Use Guide. All animal experiments were approved by the Experimental Animal Ethics Committee of Yunnan University of Chinese Medicine (Ethical Code: SYXK-K2022-0004). To measure the anti-APAP-induced-hepatotoxicity effect of STM or HTPS in mice, the mice were randomly divided into 7 groups after one week of adaptation to the environment (n = 10): (1) NC: untreated control group; (2) APAP: APAP (200 mg/kg); (3) NAC + APAP: NAC (150 mg/kg) + APAP (200 mg/kg); (4) LSTM + APAP: STM (50 mg/kg) + APAP (200 mg/kg); (5) HSTM + APAP: STM (100 mg/kg) + APAP (200 mg/kg); (6) LHTPS + APAP: HTPS (50 mg/kg) + APAP (200 mg/kg); (7) HHTPS + APAP: HTPS (100 mg/kg) + APAP (200 mg/kg). Mice were given STM, HTPS, and NAC for one week once a day, while the normal group and model group were not treated. After the last administration, the mice were fasted and given water for 12 h, then all groups were treated with APAP by intraperitoneal injection. 6 h after APAP overdose, the supernatant was collected by centrifugation at 4000 rpm for 10 min at 4 °C and the liver was kept at −80 °C for further analysis.

2.3.2. Measurement of serum ALT, AST and LDH levels in APAP-induced mice

For the detection of ALT, AST, and LDH in the serum as the biomarkers of hepatocellular injury, the activities were performed with detection kits according to the manufacturer's instructions. The liver function was evaluated 6 h after APAP overdose and the effects of STM and HTPS on impaired liver function were determined.

2.3.3. Histological analysis

The liver specimens were immersed in 10% formaldehyde for more than 24 h. The liver tissue was embedded in paraffin and cut to 4 μm, then stained with hematoxylin and eosin (H&E) reagent and observed under a light microscope.

2.3.4. Measurement of MDA, SOD, and GSH levels in APAP-induced mice

The hepatotoxicity model induced by APAP causes a redox imbalance. The liver homogenate was centrifuged at 3000 rpm for 10 min to obtain the supernatant. The content of SOD, MDA, and GSH in the liver tissues were measured according to the instructions of the reagent manufacturer.

2.3.5. Measurement of NO levels in APAP-induced mice

The liver homogenate was centrifuged at 3000 rpm for 10 min to obtain supernatant, the content of NO was measured according to the manufacturer's guidelines.

2.3.6. Measurement of TNF-α, IL-6, and IL-1β levels in APAP-induced mice

The serum levels of IL-1β, TNF-α, and IL-6 were measured by Link Bio (ELISA) kits (Proteintech, China) according to the manufacturer's protocols.

2.3.7. Western blotting

Total protein extracts from liver tissues were prepared with RIPA reagent, then centrifuged at 3000 rpm for 10 min at 4 °C to get the supernatant. The protein was determined in the supernatant with a BCA kit. According to the molecular weight of the target protein, 10 and 12% of the separation gels were prepared. For separation at 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the transfer time was 1 h. The film was completely immersed in 5% BSA-TBST for 30 min on a shaker at room temperature, then incubated with primary antibody overnight at 4 °C. Washed 3 times with Tris-buffered saline-Tween, then the film was incubated with secondary antibody for 2 h on a shaker at room temperature. Washed 5 times with TBST, the film was determined.

2.4. Statistical analysis

All experiments were independently repeated at least three times. Data were expressed as mean ± standard deviation. All analyses were computed using SPSS version 16.0 statistical software, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. STM or HTPS alleviated apoptosis in APAP-induced L-O2 cells

The effect of STM or HTPS on APAP-induced apoptosis in L-O2 cells was detected by CCK-8 kit (Fig. 2A). The results showed that the survival rate of APAP-induced L-O2 cells was significantly decreased compared with NC group. Upon treatment with APAP and STM or HTPS, the survival rate of APAP-induced L-O2 cells increased significantly, compared with APAP group. In addition, the results of Annexin V-FITC/PI staining (Fig. 2B) showed that the apoptosis rate of APAP-induced L-O2 cells decreased gradually with the concentration increase of STM and HTPS.

3.2. STM or HTPS alleviated hepatotoxicity in APAP-induced L-O2 cells

As shown in Fig. 3A–C, ALT and AST levels were significantly increased in APAP-induced L-O2 cells, compared with NC group. STM or HTPS treatment significantly prevented the increase in ALT, AST levels in APAP-induced L-O2 cells compared with APAP group.

3.3. STM or HTPS decreased oxidative stress and inflammation in APAP-induced L-O2 cells

The levels of SOD and MDA were measured in APAP-induced L-O2 cells (Fig. 4A–D). The results showed that the SOD level in the APAP group was decreased, and the MDA level was increased. Treatment with STM or HTPS restored SOD levels and restrained the increase of MDA levels. These results demonstrated that STM or HTPS reduced the oxidative stress injury in a concentration-dependent manner in APAP-induced L-O2 cells. The results indicated that the IL-6 and TNF-α levels in the APAP group were increased, while the treatment with STM or HTPS decreased the levels of IL-6 and TNF-α. These results demonstrated that STM or HTPS reduced the inflammation in a concentration-dependent manner in APAP-induced L-O2 cells.

3.4. STM or HTPS alleviated liver injury in APAP-induced mice

Serum LDH, AST, and ALT were analyzed for the evaluated STM or HTPS effects on mice liver function after APAP overdose. As shown in Fig. 5A–C, the levels of ALT, AST, and LDH increased 6 h after APAP administration compared to the NC group, showing the existence of liver injury. The levels of ALT, ATL, and LDH decreased in a dose-dependent manner in the groups pretreated with STM or HTPS for 7 days, compared to the APAP group. Notably, HTPS showed better therapeutic effects than STM as indicated by the resulting statistical significance. In addition, the results of histological evaluation of the liver also confirmed the efficacy of STM or HTPS in alleviating APAP-induced liver injury such as tissue edema, degeneration, necrosis and inflammatory changes (Fig. 5D).

3.5. STM or HTPS decreased oxidative stress in APAP-induced mice

In order to evaluate the antioxidant potential of STM or HTPS in APAP-induced mice, analyses were performed to determine the levels of MDA, SOD, and GSH. Results showed that pretreatment with STM or HTPS improved the increase of MDA level and the decrease of GSH and SOD levels caused by APAP (Fig. 6A–C). These results suggest that STM or HTPS pretreatment was effective at protecting against oxidative damage caused by the APAP overdose.

3.6. STM or HTPS attenuated inflammation in APAP-induced mice

APAP intoxication leads to liver disorders, which result in inflammatory state. The levels of TNF-α, IL-1β, IL-6 and NO in mice and the effects of STM or HTPS pretreatment were evaluated by Kits for detection. As shown in Fig. 7A–D, the expressions of TNF-α, IL-1β, IL-6, and NO increased in the APAP group compared to the NC group, and this was statistically significant. STM or HTPS pretreatment

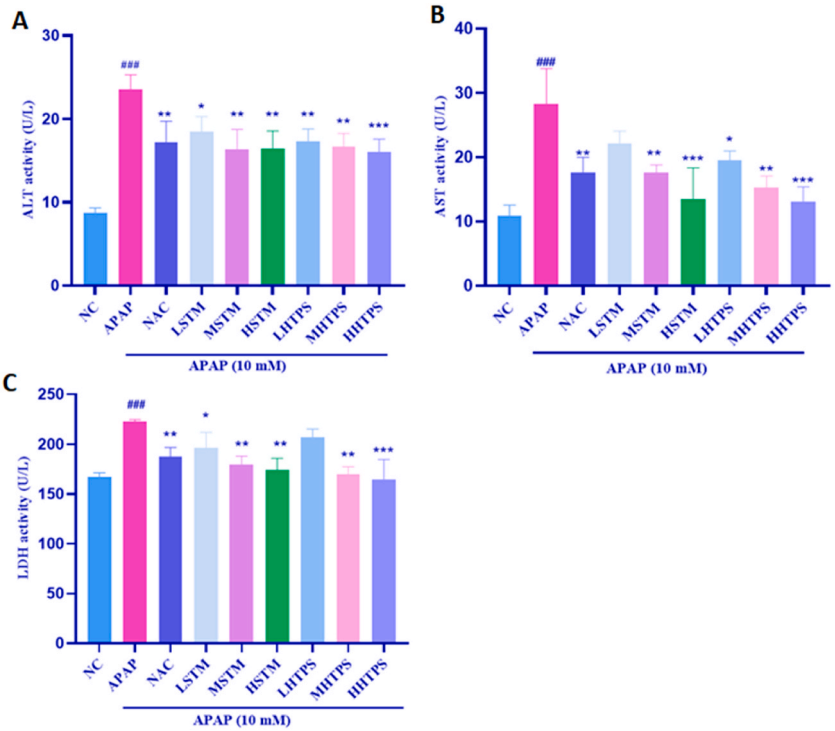

Fig. 3. STM or HTPS alleviated hepatotoxicity in APAP-induced L-O2 cells. ALT (A), AST (B) and LDH (C) in L-O2 cells were detected according to the manufacturer's instructions. The values are given as the mean ± SD (n = 5). $^{###}p < 0.005$ versus NC group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ versus APAP group.

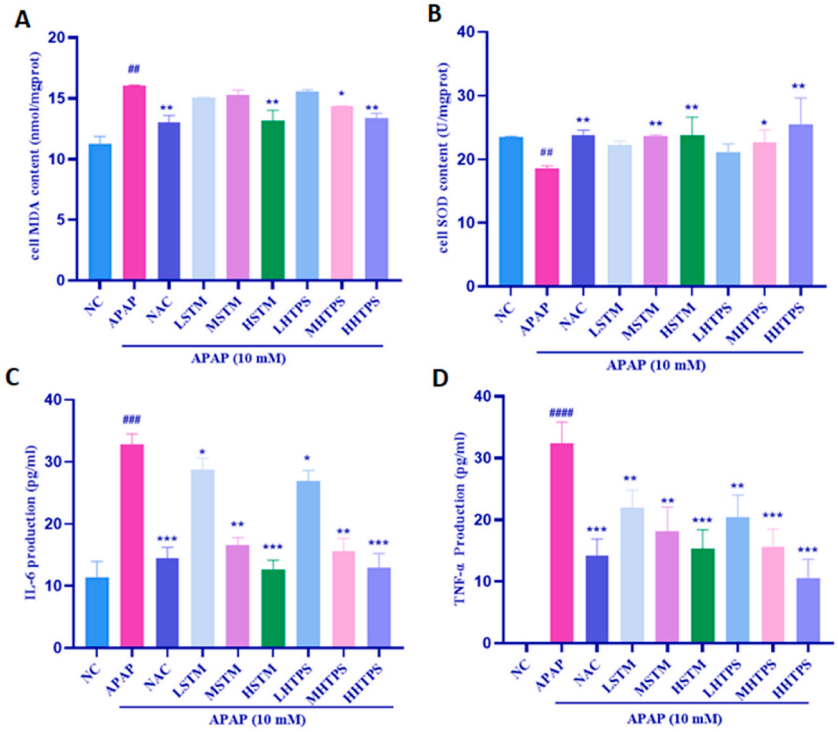

Fig. 4. STM or HTPS decreased oxidative stress and inflammation in APAP-induced L-O2 cells. MDA (A), SOD (B), IL-6 (C) and TNF-α (D) in L-O2 cells supernatant were detected according to manufacturer's protocol. The values are given as the mean ± SD (n = 5). $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.005$, $^{\#\#\#\#}p < 0.001$ versus NC group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ versus APAP group.

reduced the levels of TNF-α, IL-1β, IL-6, and NO compared to the APAP group. The results were statistically different.

3.7. STM or HTPS regulated the Nrf-2/HO-1/NF-κB signaling pathways in APAP-induced mice

Nrf-2 has been a potential target for the treatment of a variety of liver diseases [16,17]. HO-1 is an important cellular antioxidative enzyme, whose gene expression was reported to be regulated by Nrf-2 [18]. Results demonstrated that there is a decrease in Nrf-2 as well as in HO-1 activity in the APAP group compared to the NC group (Fig. 8A). Pretreated with STM or HTPS, Nrf-2 and HO-1 in APAP-induced mice were significantly restoredand the quantitative results of protein showed that the contents of Nrf-2 and HO-1 increased significantly compared with those of APAP group (Fig. 8B). NF-κB plays critical role in the regulation of inflammatory genes. In this study, the expression of the phosphorylation of IκBα and NF-κB indicate that STM or HTPS regulated the NF-κB signaling pathway after APAP overdose exposure. There was an increase in p–NF–κB and p- IκB activity in the APAP group compared to the NC group. The STM or HTPS pretreated groups had significantly reduced p-IκB and p–NF–κB activity compared with APAP group, showing that STM or HTPS have an effect on the modulation of NF-κB pathway (Fig. 8C). The quantitative results of protein showed that the content of p-IκB and p–NF–κB was significantly lower than APAP group (Fig. 8D).

4. Discussion

In this study, the effects of different doses of STM or HTPS on APAP-induced hepatotoxicity model were evaluated *in vitro* and *in vivo*. We evaluated L-O2 cells apoptosis, liver function enzymes, changes in hepatic parenchyma, activities of MDA, SOD and GSH in antioxidant defense system, changes of inflammatory factors TNF-α, IL-6, IL-1β and NO, and protein expression of Nrf-2 and NF-κB pathways.

Studies show that inflammatory mediators and oxidative stress play an important role in the process of hepatotoxicity induced by APAP [19,20]. Lipid peroxidation and oxidative stress damage lead to abnormal cell function, which eventually leads to cell death or apoptosis [21,22]. In this study, the administration of STM or HTPS significantly inhibited the apoptosis of L-O2 cells induced by APAP. ALT, AST, and LDH are usually the markers of liver injury [23,24], hence, the APAP intoxication leads to liver dysfunction, characterized by an increase of ALT, AST and LDH [25]. The present study showed that APAP significantly increased the activities of ALT/AST/LDH in mice or L-O2 cells. ALT/AST/LDH levels were dramatically decreased by the treatment of STM or HTPS. These results indicate that STM or HTPS protected the L-O2 cells apoptosis and alleviated APAP-induced hepatotoxicity. It is worth noting that the analysis of serum LDH, AST and ALT in mice shows that HTPS is better than STM in treating APAP-induced liver injury, which is statistically significant ($p < 0.05$).

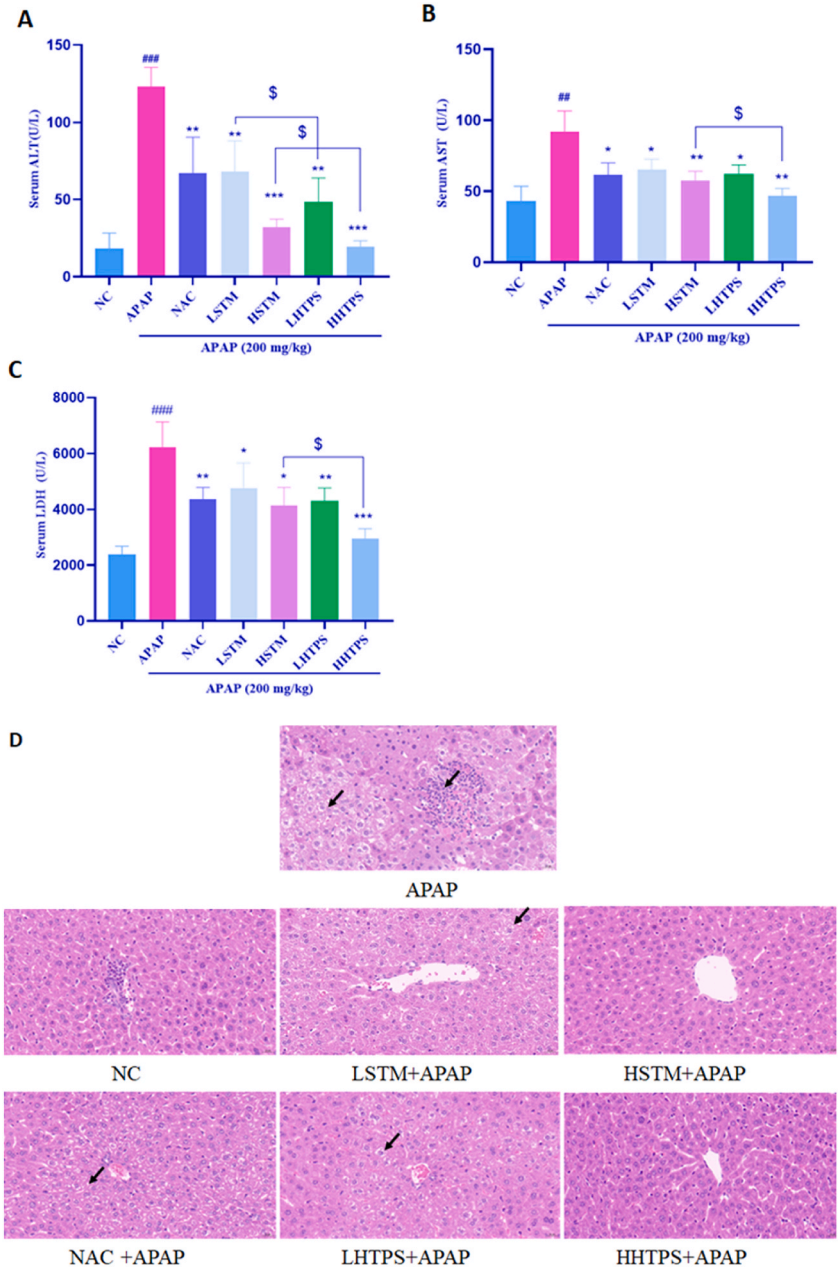

Fig. 5. STM or HTPS alleviated liver damage in APAP-induced mice. ALT (A), AST (B), and LDH (C) were detected in mice. The liver sections were stained with hematoxylin-eosin for the evaluation of liver pathological changes (400× magnifications) (D). Areas of liver inflammatory changes were marked by arrows, no obvious inflammatory changes were found in the NC group, APAP group has a large number of watery degeneration with hepatocyte necrosis. The values are given as mean ± SD (n = 10). $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.005$ versus NC group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ versus APAP group; $^{\$}p < 0.05$ versus STM group.

GSH is a critical antioxidant for NAPQI in APAP hepatotoxicity [26], however, once GSH is deficient in the liver, the accumulation of NAPQI triggers a series of toxic reactions, causing oxidative stress and cell apoptosis [27,28]. APAP-induced hepatotoxicity is characterized by enhanced lipid peroxidation and decreased functioning of antioxidant defense systems [29]. SOD is responsible for turning the superoxide anion into hydrogen peroxide [30], while MDA is a lipid peroxidation marker. MDA and SOD are often used as markers to measure oxidative stress [31]. The oxidative stress-induced cells generate a large amount of MDA, accompanied by the consumption of antioxidant enzyme SOD [32]. Our study showed that STM or HTPS significantly reduced MDA level, restored the antioxidant capacity of SOD, and reduced the consumption of GSH in the APAP-induced hepatotoxicity model. The results showed that STM or HTPS attenuated APAP-induced hepatotoxicity by inhibiting oxidative stress.

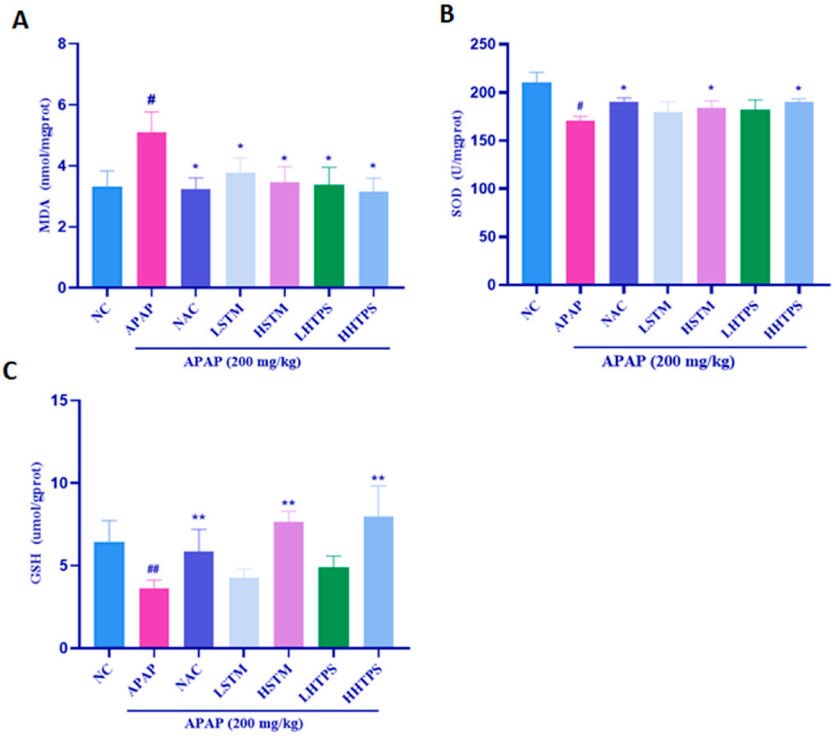

Fig. 6. STM or HTPS decreased oxidative stress in APAP-induced mice. Determination of MDA (A), SOD (B) and GSH (C) levels in liver. The values are given as the mean ± SD (n = 10). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus NC group; $^{*}p < 0.05$, $^{**}p < 0.01$ versus APAP group.

Nrf-2 is a key intracellular antioxidant factor that regulates oxidative stress [33], and the effect of Nrf-2 on antioxidant stress has been fully demonstrated [34,35]. Cellular injury causes Nrf-2 nuclear accumulation and subsequent activation of downstream genes, including HO-1 [36]. HO-1 is an enzyme that acts as an antioxidant and possesses anti-inflammatory properties [37]. Ginsenoside Rg1 protected APAP-induced liver injury by upregulating Nrf-2 [38], which was consistent with our results that the administration of STM or HTPS activates nuclear factor Nrf-2 and upregulates the expression of HO-1 to play a protective role in liver injury, and starts the defense mechanism of endogenous oxidative stress, indicating that Nrf-2/HO-1 pathway is the main regulatory pathway for STM or HTPS to inhibit APAP-induced hepatotoxicity.

Inflammatory response plays an important role in APAP-induced hepatotoxicity [39]. Previous studies have shown that the levels of inflammatory cytokines TNF-α, IL-6, IL-1β [40] and NO [41] in APAP-induced liver injury are significantly increased. STM or HTPS treatment significantly decreased the levels of TNF-α, IL-6, IL-1β, and NO in APAP-induced mice. NF-κB mediates the transcription of various genes, recruited by inflammatory cells, and triggers a series of inflammatory reactions [42], further regulating the levels of TNF-α, IL-6, and IL-1β [43]. Upon activation of NF-κB, the NF-κB subunit p65 was promoted via the phosphorylation and degradation of IκBα [44]. The NF-κB activation would increase the phosphorylation of NF-κB, IκBα levels [45]. The present study has shown that due the treatment of STM or HTPS, the inflammatory level induced by APAP and the phosphorylation of NF-κB/IκBα were inhibited. Inhibiting the activation of NF-κB pathway may be the main mechanism of STM or HTPS protecting APAP-induced anti-inflammatory injury.

5. Conclusion

Our results confirm the preventive effect of STM or HTPS on APAP-induced toxicity *in vivo* and *in vitro*. The protective effect of STM or HTPS on APAP-induced hepatotoxicity is mainly through inhibiting apoptosis and regulating Nrf-2/NF-κB signaling pathway. Notably, HTPS have better activity against APAP-induced hepatotoxicity than STM, which implied that HTPS might be the effective components of QYD tablets, rather than STM. It is worthy of further detailed study on the chemical composition of HTPS to discover potential drug for treating liver diseases.

Author contribution statement

Qian Zhou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Qixiu Zhou: Rui Xia: Peng Zhang: Performed the experiments.

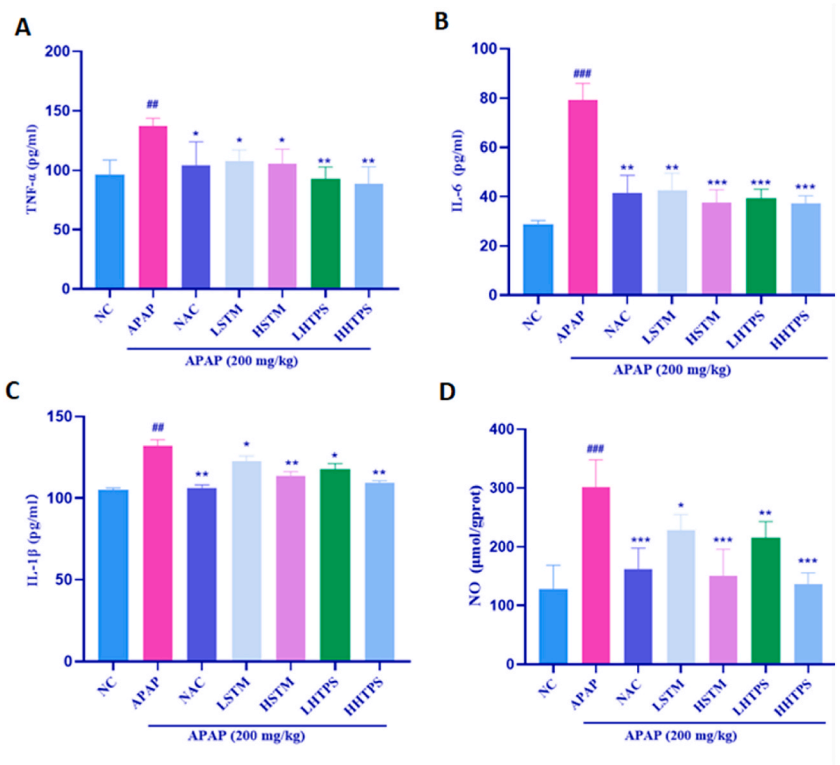

Fig. 7. STM or HTPS attenuated inflammation in APAP-induced mice. Determination of TNF-α (A), IL-6 (B), IL-1β (C) and NO (D) levels in mice. The values are given as mean ± SD (n = 10). $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.005$ versus NC group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ versus APAP group.

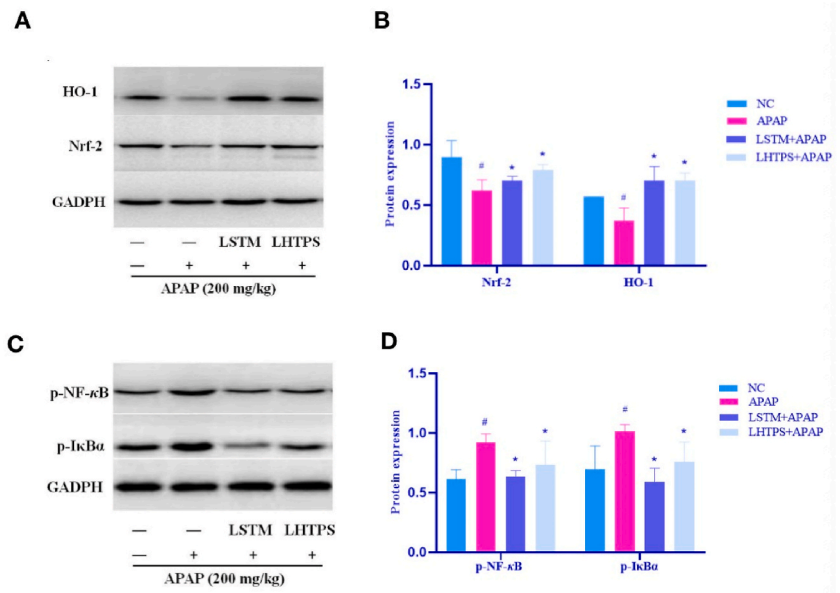

Fig. 8. STM or HTPS regulated the Nrf-2/HO-1/NF-*κB* signaling pathways in APAP-induced mice. The protein levels of Nrf-2 and HO-1 in the liver tissue were determined (A), The quantitative results of Nrf-2 and HO-1 protein (B), The protein levels of p–NF–κB, p-IκBα in liver tissue were determined (C), (The quantitative results of p-IκB and p–NF–κB protein in liver (D). The values are given as means ± SD (n = 3). $^{\#}p < 0.05$ versus NC group; $^{*}p < 0.05$ versus APAP group.

Yanqing Xie: Zhuya Yang: Wenhong Tan: Afsar Khan: Zhihong Zhou: Lu Liu: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18746.

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