The Relationship Between PI3K / AKT Pathway and Intestinal Flora Confirms the Liver-Gut Axis Theory to Evaluate the Mechanism of POP Inhibition Of APAP-Induced Acute Liver Injury in Mice

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Background: *The purpose of this study was to investigate the relationship between PI3K / AKT pathway and intestinal flora, and the liver-gut axis theory was confirmed to evaluate the mechanism of POP inhibition of APAP-induced acute liver injury in mice.*

Methodology*: Liver tissue H&E, TUNEL, Hoechst 33,258, immunofluorescence staining, western blot method was used and intestinal flora were tested to comprehensively evaluate the protective mechanism and therapeutic effect of POP on liver injury induced by single injection of APAP (250 mg / kg) in mice.*

Results: *POP could increase the proportion of Lactobacillus and decrease the abundant of norank_o_Clostridiales and Prevotella compared with APAP induc tion liver injury in the intestine of mice. POP pretreatment could reverse GSH depletion and CYP2E1 overexpression, reduce the expression of MDA and 4-HNE, and decrease the contents of ALT, AST, TNF-α and IL-1β, while the related proteins of PI3K / AKT signaling pathway are also close to the level of normal control group. POP pretreatment was significantly to reduce APAP-induced liver tissue apoptosis, necrosis and inflammatory infiltration.*

Conclusion: *The PI3K/AKT pathway-mediated Bax/Bcl-2 and NF-κB signal cascade was blocked and interfered by POP, which was shown by protecting the structure of intestinal flora and repairing the mice intestine mucosa to suppress the oxidative stress of liver tiss, improve the inflammatory response and reduce liver cell apoptosis and necrosis.*

BACKGROUND

Modern pharmacological reports suggest that the liver and intestine are a group of important organs that are closely related.[1] About 70% to 80% of the blood supply in the liver comes from the portal vein, which mainly collects blood from the superior mesenteric vein and sub mesenteric vein. The "gut-liver axis" theory is mainly reflected in the bacterial translocation after the intestinal barrier is damaged, then endotoxin enters the portal vein system,[2] activates the liver kupffei cells, and then releases a series of inflammatory factors, causing liver immune damage and inflammatory response.[3] On the other hand, the interaction and influence of various cytokines and inflammatory mediators also induce intestinal mucosa and distant organ damage.[4] In recent years, studies on the intestinal-liver axis and chronic liver disease have confirmed the complex interrelationship between the

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intestine and the liver. For example, animal studies on the bacterial translocation and dynamic changes of the intestinal flora in the early stages of acute liver injury suggest that increased intestinal permeability and translocation of intestinal bacteria may occur.[5] Although the pattern of changes in the intestinal flora caused by liver injury varies form depending on different condition, it still shows excessive growth of intestinal bacteria and abnormal intestinal flora. Excessive use of Acetaminophen (APAP) will cause acute liver damage.[6,7] At the therapeutic dose, APAP is mostly metabolized to non-toxic compounds by phase II metabolic enzymes such as uridine diphosphate (UDP) glucuronyl transferase (UGT) and sulfate transferase (SULT), which is excreted with urine. Only a small portion of about 5-9% APAP is metabolized by cytochrome P450 enzymes (CYPs),[8] and is mainly converted by CYP2E1 into the highly reactive intermediate metabolite N-acetophenimidinone (NAPQI).[9,10] Generally, NAPQI is rapidly detoxified by binding to glutathione (GSH). However, when APAP is excessive, it will lead to saturation of phase II metabolic enzymes, and the excessive production of NAPQI will consume GSH, and the remaining NAPQI will react with cell membrane molecules, especially with covalent binding of sulfhydryl groups in mitochondrial proteins,[11] Which in turn leads to mitochondrial oxidative stress and dysfunction, and eventually causes hepatocyte necrosis. Polygonatum odoratum polysaccharides (POP) is a kind of polysaccharides was isolated from the Polygonatum odoratum (Mill.) Druce which a traditional Chinese herb used as a preventative measure against diabetes,[12] hyperlipidemia, and some cancers.[13,14] POP have been confirmed to be the primary contributor to the majority of observed benefits,[15] such as anti-obesity,[16] hypoglycemic,[17] prevent liver damage,[18] regulate intestinal flora[19,20] and other pharmacological effects. Although POP had been reported to present the strong ability of protecting the liver in the CCl4-induced liver oxidative injury, few studies have focused on the exact molecular mechanism through that POP regulated intestinal flora to improve APAP-induced liver toxicity in vivo. Thus, the present study aimed to assess the effects of POP as a hepatoprotective candidate in reducing hepatic injury following APAP treatment

MATERIALS AND METHODS

Chemical compounds and reagents

POP of 98% purity was purchased from Shaanxi Tianrui Biotechnology Co. (Xi'an, China). APAP was purchased from Yan sheng Biotech Co., Ltd. (Shanghai, China). The rabbit monoclonal anti-mouse AKT, p-AKT, Bax, Bcl-2, CYP2E1, 4-HNE, GAPDH, IKKα, IKKβ, p-IKKα/β, I-κBα, p-I-κBα, PI3K, p-PI3K, NF-κB p65 and p-NF-κB p65 antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). TNF-α and IL-1β ELISA kits were purchased from the R&D company (Minneapolis, MN, USA). Hematoxylin and eosin (H &E) dyes, ALT, AST, MDA, and GSH assay kits were purchased from Nanjing Jincheng Institute of Biotechnology (Nanjing China). Hoechst 33,258 dye kit was bought from Beyotime Biotechnology Co., Ltd. (Shanghai, China). TUNEL apoptosis detection kits were bought from Roche Applied Science (Shanghai, China). DyLight 488-SABC immunofluorescence staining kit was purchased in BOSTER Biological Technology Co., Ltd. (Wuhan China). The remaining reagents were analytical pure, and provided by Beijing Chemical Works (Beijing, China).

Animals and treatment

Male ICR mice (weighting 20-25 g, Certificate No. JLUN (JI) 2019-0005) were purchased from JILIN University (Changchun, China). All mice were housed in plastic-bottomed cages with reticulate stainless-steel covers under controlled light conditions (12-h light-dark cycle). All animal protocols were strictly in accordance with the Regulation on Management of Experimental Animals issued by the Ethical Committee for Laboratory Animals at Jilin Agricultural Science and Technology College (Permit No. ECLA-JASTC-18065).

After one week acclimatization, mice were randomly assigned to 4 groups (n=6): (1) Normal control group treated with CMC-Na, (2) APAP-treated group (250 mg/kg), (3) APAP/POP group (250/150 mg/kg), (4) APAP/POP-treated group (250/300 mg/kg). Mice of group 3 and 4 were pretreated by gavages with POP (150, 300 mg/kg per day) for 7 days continuously.

On the seventh day, mice of groups 2, 3 and 4 were all injected with a single dose of APAP (250 mg/kg) after pretreatment of POP for 1 h. The experiment ended after 24 h, when fecal samples of all mice were collected, immediately transferred to pre-labeled microcentrifuge tubes, and frozen in liquid nitrogen until further processing. All mice were killed immediately by cervical dislocation, and blood was collected and centrifuged at 3000g for 10 min. Serum was taken from the supernatant and stored at −80 ℃ until analysis. In the meantime, liver tissues were collected and weighted to calculate the organ indices. Then, part of the liver samples was fixed in 10% formalin solution (m/v) for at least 24 h, and embedded in paraffin for tissue sections, the remaining tissue was rapidly frozen in liquid nitrogen and stored at −80 ℃ preparation of homogenate.

Biochemical marker assay

The liver tissue of the mice was re-suspended in PBS and then the samples were centrifuged at 3000 rpm for 10 min at room temperature depending on the kit protocol. The supernatant was collected for subsequent experiment. And serum was obtained by plasma centrifugation at 3000 rpm for 10 min at room temperature. The activity of AST, ALT, GSH and MDA were quantified by commercially available kits as mentioned above. The contents of endotoxin, TNF-α and IL-1β in serum were determined using ELISA assay kits according to the manufactures' protocols.

H&E, TUNEL, Hoechst 33,258, CYP2E1 and 4-HNE staining

The liver tissues immersed in 10% formaldehyde for more than 24 hours were then embedded in paraffin and made into sections (Leica RM2125 RTS). The 5 μm-thickness sections were stained with H&E, Hoechst 33,258 and TUNEL respectively according to the manufactures' protocols for verifying changes of the liver tissues including hepatocyte necrosis, cell apoptosis and central vein congestion. The protein expression of 4-HNE and CYP2E1 in APAP-induced hepatotoxicity were detected by immunofluorescence staining 4-HNE (1: 200) and CYP2E1 (1: 200) as previously described with minor modifications.[19, 20] The degree of liver apoptosis was evaluated by using Image-Pro plus 6.0 software (Media Cybernetics, Maryland, and USA) through quantifying the fragmented and condensed staining.

Western blot analysis

Liver tissues (200 mg) were homogenized in 2 mL RIPA buffer, and protein concentration was determined with BCA protein assay kit (P0011, Beyotime Biotechnology, Shanghai, China). Samples were denatured at 100 °C for 5 min and stored at −80 °C until needed for western blotting. Equal amounts of protein (20 μg/lane) were resolved by 10% SDS-polyacrylamide gel electrophoreses (SDS-PAGE) and transferred to nitrocellulose membrane. The following antibodies were used to hybridize with PVDF membrane: AKT, p-AKT, Bax, Bcl-2, CYP2E1, 4-HNE, GAPDH, IKKα, IKKβ, p-IKKα/β, I-κBα, p-I-κBα, PI3K, p-PI3K, NF-κB p65, p-NF-κB p65, GRP78 and TLR4, and then were quantified by a densitometric analysis (Bio-Rad Laboratories, Hercules, CA, USA).

Analysis of the composition of the intestinal flora of mice

After 24 hours of intraperitoneal injection of APAP, the feces of each group of mice were collected, placed in a 10 mL dry sterilized centrifuge tube, and stored in a refrigerator at -80 °C for testing. The collected mouse stool samples were sent to Shanghai Meiji Biomedical Technology Co., Ltd. for 16SrRNA sequencing. The sequencing data was compared through the bioinformation cloud i-singer platform, the species abundance of each sample was counted at different classification levels, and the composition of the intestinal bacterial community was analyzed.

Statistical analysis

All data were presented as means ± SD. Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA), and significance was verified via one-way ANOVA followed by the

Student's t-test. The p values < 0.05 or 0.01 were taken as the criterion for statistical significance.

RESULTS

Effects of POP on organ indices and serum

After APAP 250 mg/kg administration, the body weight of mice was significantly lower than CK and POP pretreatment groups, while the spleen and liver indices were much higher than normal and POP groups ($p < 0.05$) (Table 1), which indicated that POP showed a positive effect on mice liver and spleen.

The interested phenomenon was also confirmed by ALT and AST data in serum which were used to determine the degree of liver damage of the mice in APAP-induced and POP-pretreatment groups. As shown in Figure. 1A and B, compared with the control group, the levels of ALT and AST were significantly increased in the serum of mice after APAP stimulation ($p < 0.01$), however, were reduced in the serum of the two POP-pretreatment mice ($p<0.01$), and the better effect was detected in the high-dose POP-pretreatment group (300 mg/kg).

Table 1: Effects of POP on body weights and organ indices in mice. (n=6, Mean ± S.D)

Groups	Dosage (mg/mL)	Body weight		Liver index (mg/g)	Spleen index (mg/g)
		Initial (g)	Final (g)		
Normal	0	21.48±1.14	24.38±0.83	38.35±1.22	3.26±0.7
APAP	250	21.48±0.87	22.23±0.75*	41.36±1.58*	4.94±1.8**
APAP+POP	150	21.43±1.23	23.68±0.69#	39.47±0.97#	3.91±1.6
	300	21.45±1.13	23.87±0.88#	38.67±1.14#	3.24±1.2#

(*P<0.05, **P<0.01 vs. Normal group; # P<0.05, ## P<0.01 vs. APAP group)

POP regulates liver oxidative stress

GSH levels of liver were significantly reduced in the APAP-induced group compared with the control group ($p <0.05$), while the levels of GSH were increased in POP-pretreatment group Figure. 1C. The MDA levels presented in Figure. 1D were higher than APAP group ($p <0.05$), but it was alleviated in both POP-administered groups, indicating that APAP caused oxidative stress injury can be inhibited by POP ($p <0.05$).

In order to further investigate whether the POP was able to regulate the APAP-induced liver oxidative stress injury, the expression of CYP2E1 and 4-HNE in liver tissue were detected by using immunofluorescence technique after 24 hours of APAP-induced. Stronger CYP2E1 and 4-HNE fluorescence intensities were visually displayed in Figure. 1E and F, but this phenomenon was after 7 days of POP-pretreatment (150 and 300 mg/kg), while, the same situation was further verified by analysis of the data in Figure. 1G and H ($p < 0.01$). The results demonstrated that POP could at least partially prevent APAP-induced liver injury by inhibiting oxidative stress.

POP attenuates necrosis of hepatocytes

As showed in Figure.2A, hepatocyte cavitation was extremely prominent throughout the entire visual field of the microscope in APAP-induced liver injury group compared with normal control group in which full hepatocytes and clear hepatic lobes in the liver tissue could be observed without showing histopathological changes. The microstructure of liver tissue in POP-pretreated (300 mg/kg) high-dose group was similar to the CK group, while

hepatocyte cords were not clear enough, and hepatocytes were slightly cavitated in low-dose POP-pretreatment group (150 mg/mL),
which was basically normal. The necrotic score of histopathological changes was also clearly shown in Figure 2B which presented dose-dependent manner (150 or 300 mg/kg/day, 1 week)
indicated that POP-pretreatment could reduce APAP-induced liver pathological changes ($p < 0.01$).

Figure 1. POP protected liver against emergency injury. (A) ALT and (B) AST levels in serum; (C) GSH and (D) MDA level in liver; (E) CYP2E and (F) 4-HNE immunofluorescence images; The relative fluorescence intensity of (G) CYP2E1 and (H) 4-HNE. The immunofluorescence methods were used to assess the the expression level of CYP2E1 and 4HNE (Green) in tissue sections isolated from different groups. Representative immunofluorescence images were taken at 400×. (mean ± S.D., n = 6. *p < 0.05, **p < 0.01 vs. normal group; #p < 0.05, ##p < 0.01 vs. APAP group).

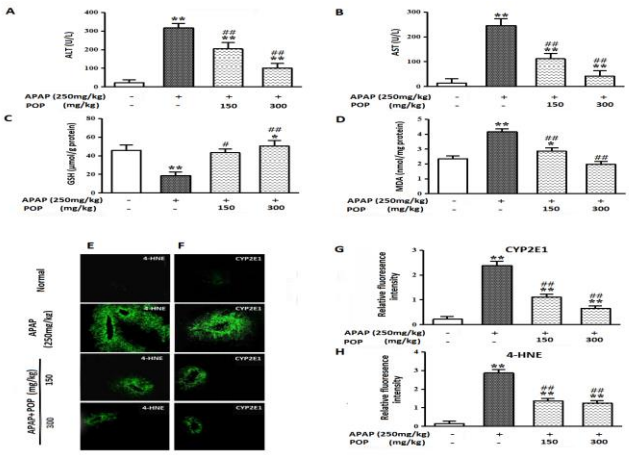
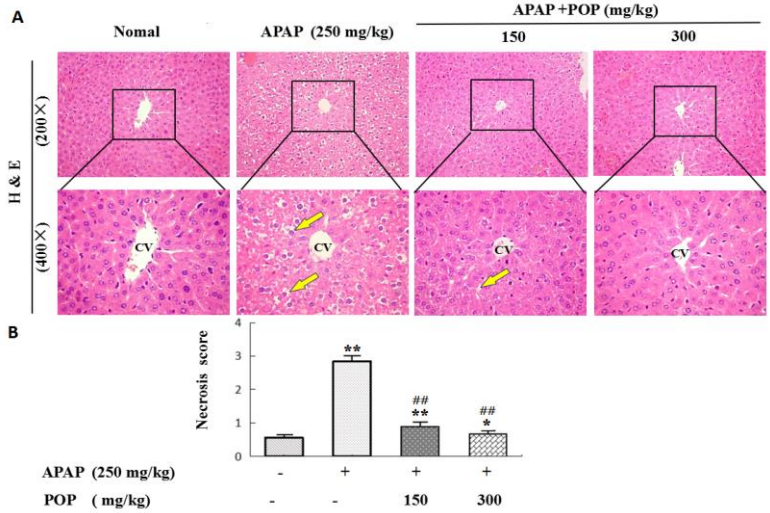


Figure 2. POP attenuates necrosis of hepatocytes in APAP-induced liver injury mice. (A) Hematoxylin-eosin (H&E) staining of liver tissues (200×, 400×). Yellow arrows indicate hepatocyte apoptosis cavitation in the H&E staining of liver tissues; (B) Necrosis score of liver tissue in different groups. 0 = no damage, 1 = 0–10%, 2 = 11%–25%, 3 = 26%–45%, 4 = 46%–75%, 5 = > 75%. All data were expressed as mean ± S.D., n = 6. *p < 0.05, **p < 0.01 vs. CK; #p < 0.05, ##p < 0.01 vs. APAP group.



POP inhibits hepatocyte apoptosis

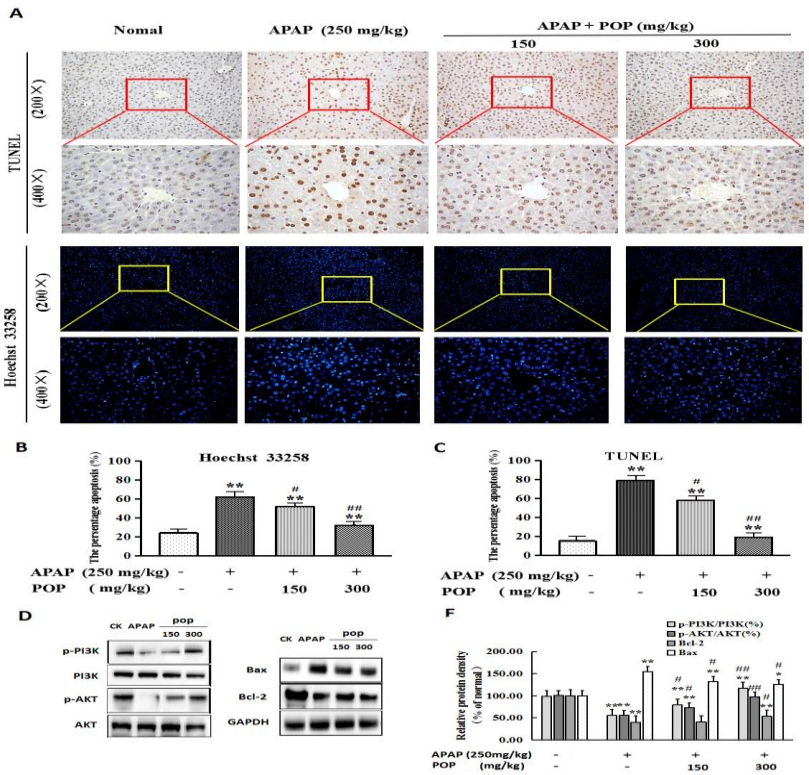
The data showed that almost all hepatocytes in the APAP group displayed nuclear division and coagulation Figure. 4A and B, but it was prevented by POP-pretreatment in which a small number of hepatocytes nuclear fragmentation and condensation were observed in POP (150 mg/kg) pretreatment group, while normal hepatocyte contours and the regular homogeneous fluorescence intensity were exhibited in POP (300 mg/kg) pretreatment group ($p < 0.01$). Tunel staining was also used to investigate apoptosis degree of hepatocyte nuclei in liver tissue, and the result showed that almost no positive cell expression was detected in control group, but larger number of TUNEL-positive cell was expressed in APAP group.

This phenomenon was successfully reversed by POP-pretreatment and also appeared dose-dependent manner ($p < 0.01$) Figure. 3A and C.

To explore the effect of POP-pretreatment on hepatocyte apoptosis, PI3K/AKT signaling pathway related proteins such as pro-apoptotic factor Bax and anti-apoptotic factor Bcl-2 were detected by Western blot.

The data Figure. 3D and E presented a significant decrease in the expression of p-PI3K and AKT after APAP-induced ($p < 0.05$ or $p < 0.01$), but were increased in POP-pretreatment groups (150 and 300 mg/kg). The expression of Bax was increased and the expression of Bcl-2 was decreased in liver tissue after one dose APAP injection (250 mg/mL), however, the degree of hepatocyte apoptosis by APAP-induced could be reduced by POP-pretreatment at 150 and 300 mg/kg ($p < 0.05$ or $p < 0.01$).

Figure 3. Analysis of POP-regulated apoptosis. (A) Liver tissue was stained with TUNEL (200 ×, 400 ×) and Hoechst 33,258 (200 ×, 400 ×). In TUNEL (400 ×), yellow arrows indicate apoptotic cells. Percentage of Hoechst 33,258 (B) and TUNEL (C) positive cells by image analyzer. (D) The protein expressions of p-PI3K, PI3K, p-AKT, AKT, Bax and Bcl-2. (E) Quantification of relative protein expression of p-PI3K, PI3K, p-AKT, AKT, Bax and Bcl-2. Values represent mean ± SE (n =6). *p < 0.05, **p < 0.01 vs. CK; #p < 0.05, ##p < 0.01 vs. APAP group.



POP inhibits inflammation and endotoxin results

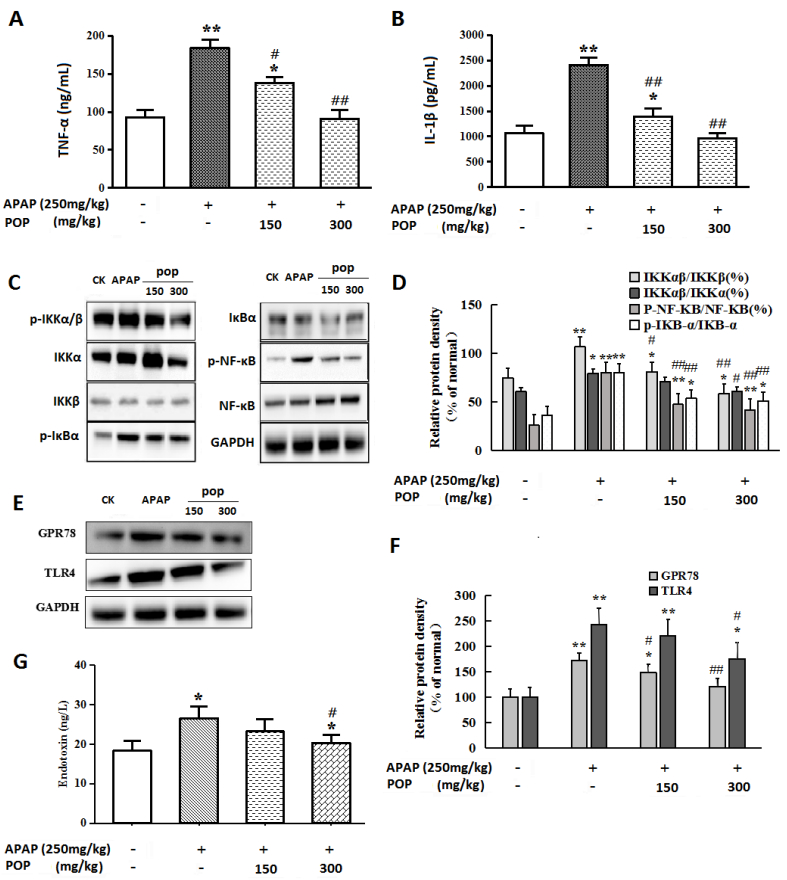
To verify the correlation between APAP-induced liver toxicity and inflammatory response, we measured the levels of the TNF-α, IL-1β and endotoxin in serum. As shown in Figure. 4A, B and C, compared with the normal control group, the levels of TNF-α, IL-1β and endotoxin in serum of APAP-induced mice were increased ($p < 0.01$). However, in mice treated with POP, levels of TNF-α, IL-1β and endotoxin were significantly reduced ($p < 0.05$ or $p < 0.01$) respectively.

NF-κB has been reported [3,21] may regulate the transcription of pro-inflammatory cytokines. The expression of phosphorylated and non-phosphorylated forms of NF-κB signaling pathway-related proteins in liver tissues were analyzed by Western blot to evaluate liver protection-related signaling molecules.

The expression levels of GPR78, TLR4, p-IKKα/β, p-NF-κB and p-IκBα in liver tissues were generally increased due to APAP induction, but all protein data showed a downward trend after pretreatment with POP (150 and 300 mg/kg).

Therefore, it was shown that POP can inhibit the activation and expression of the NF-κB signaling pathway and significantly down-regulate the expression levels of these proteins ($p < 0.05$ or $p < 0.01$).

Figure 4. Effects of POP inhibits inflammation and endotoxin in mice with liver injury induced by APAP. (A) TNF-α levels. (B) IL-1β levels. (C) Endotoxin levels. (D) The protein expressions of phosphorylated and total IKKα, IKKβ, IκBα and NF-κB. (E) Quantification of relative protein expression of IKKα, IKKβ, IκBα and NF-κB. (F) The protein expressions of GPR78 and TLR4. (G) Quantification of relative protein expression of GPR78 and TLR4. Data are expressed as mean ± SD (n = 6). *p < 0.05, **p < 0.01 vs. CK; #p < 0.05, ##p < 0.01 vs. APAP group.



POP Induced Structural Changes in Gut Microbiota

The results based on UniFrac analysis (PCoA) showed that the intestinal flora composition of each treatment group was clearly aggregated, while the APAP group was clearly separated from the other three groups Figure 5A. Results As expected, POP treatment increased the similarity to the overall intestinal flora composition of the NC group, indicating that POP has a substantial effect on the composition of the intestinal flora of APAP-induced liver injury. It can be seen from Figure 5C that the normal group is far away from the APAP control group, and the intestinal flora of the two groups shows a large gap in the species composition at the genus classification level; the distance between the POP (300 mg / mL) high-dose group and the APAP group were much farther, and similar to the CK group, indicating that the species composition of the intestinal flora of the POP high-dose group was close to that of the normal group.

Taxonomic analysis suggested that five major phyla were presented in fecal microbiota such as Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteri and Tenericutes Figure. 5B. The relative abundances of Firmicutes and Bacteroidetes were larger in each group, and the abundance of Firmicutes was significantly higher than that of Bacteroidetes Figure. 5D. Compared with the normal group, the proportion of Firmicutes in the intestinal bacteria of the APAP induced group was significantly reduced, while the proportion of Bacteroidetes was significantly increased. The relative abundance of Firmicutes in the intestinal bacteria of the POP administration group was significantly higher than that of the APAP induction group, while the abundance of Bacteroidetes decreased. The abundance of each phylum in POP (300mg / mL) high-dose group was close to the blank control group Figure. 5B.

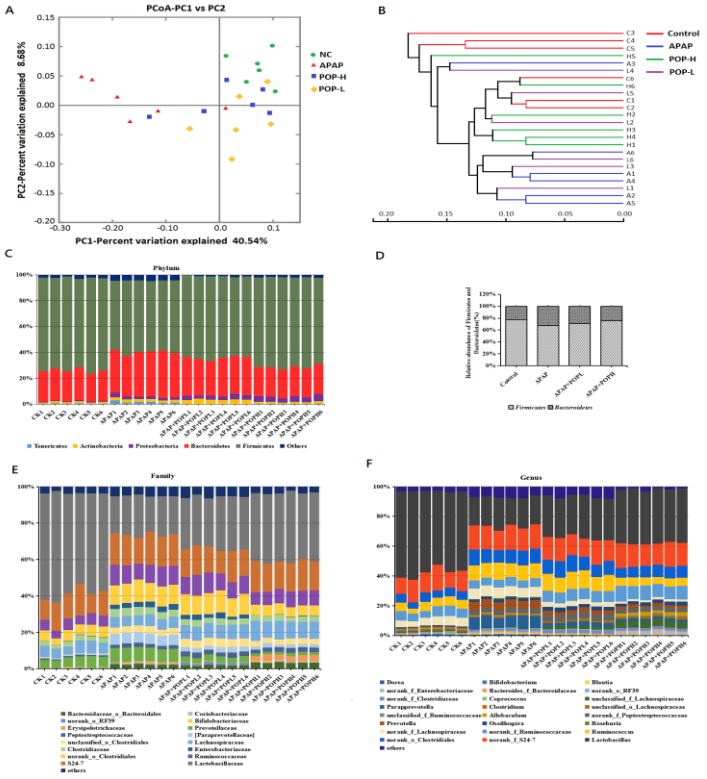
In terms of family classification Figure 5E, the composition of the intestinal flora of each group of mice is mainly represented by Lactobacillaceae, S-24-7, Ruminococcaceae, nroank_o_Clostridiales, Lachnospiraceae and Prevotellaceae. Compared with the normal group, the relative abundance of Lactobacillaceae in the APAP-induced model control group was significantly reduced, and the Compared with the APAP-induced group, the POP (150, 300 mg / mL) treatment group can significantly increase the abundance of Lactobacillaceae induced by APAP. The abundance of norank_o_Clostridiales in the high-dose POP group was close to that in the CK group, while the relative abundance of norank_o_Clostridiales in the low-dose POP group was close to that in the APAP induced group. The proportion of Prednisaceae bacteria in the intestines of mice in each POP administration group was significantly reduced.

At the genus level Figure 5E, the intestinal flora of each group of mice is mainly composed of Lactobacillus, norank_o_Clostridiales, Ruminococcus, norank_f_Ruminococcaceae, norank_f_Lachnospiraceae, Oscillospira and Prevotella. The abundance of Lactobacillus in the intestine of the APAP-induced model group was significantly lower than that of the normal group, and the relative abundance of norank_o_Clostridiales, norank_f_Ruminococcaceae, Oscillospira, and Prevotella increased significantly. Compared with the APAP induction group, the proportion of Lactobacillus in the intestine of mice in the high- and low-dose POP groups increased significantly, and the relative proportions of norank_o_Clostridiales and Prevotella decreased significantly.

DISCUSSION

POP was a kind of water-soluble polysaccharide and couldn't be absorbed by the human body. Its main function was to adjust the balance of intestinal flora, protected the intestinal mucosa, and then improved liver damage through intestinal repair. After APAP injection in mice, the liver was severely damaged, and the structure of the intestinal flora also showed variation and imbalance. The relative abundance of Lactobacillus family was significantly reduced, while the abundance of Ruminococcaceae, norank_o_Clostridiales, Lachnospiraceae and Prevotellaceae increased significantly. In this paper, it was found that the composition of the intestinal flora of POP-fed mice was very different from that of the APAP group by testing the intestinal flora of mice. The composition of flora of the high-dose

Figure 5. POP treatment regulated gut microbiota structure in APAP induced liver injury. (A) UniFrac-based PCoA plots. (B) Relative abundances of gut microbiota at the phylum level. (C) Hierarchical cluster analysis at the generic classification level. (D)Relative abundances of major phyla Firmicutes and Bacteroidetes. Relative abundances of gut microbiota at the (E) family and (H) genus levels. (n = 6).



POP group was very close to CK. The results show that the abundance of Lactobacillaceae was dominant. This similar result was consistent with the report in Reference.[21] The results showed that POP could be maintained the "gut-hepatic axis" theory by adjusting the balance of intestinal flora to repair the translocation of the intestinal barrier destroyed flora, and then prevented harmful substances such as endotoxin entering the portal vein system,[2] reducing a series of inflammatory factors released by kupffei cells protected and avoided liver immune damage and inflammation.[3] The PI3K/AKT pathway was a classic signal transduction pathway, which played an important role in various physiological and pathological processes including cell survival and differentiation, motility and apoptosis.[22,23] PI3K/AKT regulateed the transcriptional activity of the NF-κB pathway through phosphorylation and promotion of I-κB degradation.[24, 25]

After activation of NF-κB, it caused the release of downstream inflammatory factors such as TNF-α, IL-1β, etc., resulting in the body's inflammatory response that damages the liver. The transcriptional activity of the NF-κB pathway is regulated by PI3K / AKT through phosphorylation and promotion of I-κB degradation. Numerous studies have shown that the NF-κB pathway has become one of the most typical signaling pathways in various disease (Including liver and inflammatory diseases) mechanisms. This study aims to investigate the PI3K/AKT-mediated NF-κB pathway expression to further explore the mechanism by which POP inhibits APAP-induced liver injury by adjusting the intestinal flora and through the liver-gut axis mechanism. Numerous reports indicated that overdose of APAP is the most common risk factor for severe hepatotoxicity,[26] which is characterized by extensive necrosis of liver cells and rapid increase in ALT, AST, TNF-α and IL-1β,[27] GSH

depletion, overproduction of MDA, and overexpression of CYP2E1 and 4-HNE.[28] Research Data showed that pretreatment with different doses of POP reversed the exhaustion of liver GSH and overexpression of CYP2E1 induced by APAP, while POP reduced the expression of MDA and 4-HNE and decreased the content of ALT, AST, TNF-α and IL-1β in dose-dependent manner. To support the accuracy of the experimental results, H&E, Tunel, and Hoechst 33,258 staining analysis were used to clearly and intuitively observe the APAP-induced liver damage and the protective effect of POP pretreatment on liver, indicating that POP can protect liver cells from APAP-induced liver damage.

Western blot analysis was used to evaluate the signaling expression of PI3K/AKT and its downstream target proteins Bcl-2 and Bax. The results showed that the PI3K/AKT pathway APAP-induced liver injury was successfully suppressed, and the protein levels of Bax and Bcl-2 were close to those of the control group after 7 days of POP pretreatment, while POP could also reduce the expression of IKKα, IKKβ and I-κBα, and inhibited the activation of NF-κB. Therefore, the potential mechanism of POP to protect APAP toxicity may involve the regulation of intestinal flora, which prevents NF-κB signaling by inhibiting PI3K/AKT signaling pathway.

CONCLUSIONS

The PI3K/AKT pathway-mediated Bax/Bcl-2 and NF-κB signal cascade was blocked and interfered by POP. The mechanism of action may be that the mucosa of the mice intestine was protected and repaired to achieve the selectivity of further control of absorption due to POP intervention to mediate the structure of intestinal flora, while, the further results was that the oxidative stress of liver tissue was suppressed, the inflammatory response was improved, and liver cell apoptosis and necrosis were reduced. All in all, the harm to liver caused by excessive or long-term application of APAP drugs can be reduced by the application of POP pretreatment.

ABBREVIATIONS

POP, Polygonatum odoratum polysaccharides; ALF, acute liver failure (ALF); AKT, protein kinase B; I-κB, inhibitor kappa B; ALT, alanine aminotransferase; APAP, Acetaminophen; TNF-α, tumor necrosis factor-α; GSH, glutathione; 4-HNE, 4-hydroxynonenal; APAP, acetaminophen; PI3K, phosphatidylinositol-3-kinase; NF-κB, nuclear factor-kappa B; IKK, inhibitor kappa B kinase; AST, aspartate aminotransferase; IL-1β, interleukin-1β; MDA, malondialdehyde; CYP2E1, cytochrome P450 E.

Significance statement

This is the first study to demonstrate a role for Polygonatum odoratum polysaccharide (POP) in the gut-liver axis and the ability of POP to suppress liver damage induced by APAP. Our results will be of great interest to the readers of International Journal of Pharmacology, especially those involved in investigations of drug-induced liver damage, novel treatments that impact liver function, and the role of intestinal microbiota in liver function and metabolism. POP may be a useful treatment to reduce liver damage caused by excessive use of APAP and potentially other drugs that induce liver injury.

DECLARATIONS

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Conflict of interest

All authors declared that they have no conflict of interest.

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