# Research Article
# Hepato-Protective Effect of Ginsenosides from the Fruits of *Panax ginseng* Against Acetaminophen-Induced Liver Damage in Mice

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## Abstract

**Background and Objective:** Acetaminophen (APAP)-induced hepatotoxicity is a severe public health problem in western countries. Current treatment methods for poisoning are limited and novel therapeutic strategies are needed. The aim of the present study was to investigate the protective effect of ginsenosides from the fruits of *Panax ginseng* (GFG)against APAP-induced liver injury in mice and its potential molecular mechanisms of action. **Materials and Methods:** In this study, mice were orally administered with 150 or 300 mg kg$^{-1}$ of GFG for 7 consecutive days, followed by a single injection of APAP (250 mg kg$^{-1}$). Severe liver injury was observed after 24 h APAP injection and the protective effect of GFG was assessed. **Results:** The results showed that pre-treatment with GFG reduced the levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Moreover, GFG showed anti-oxidant activities characterized by reducing hepatic MDA contents and increasing hepatic SOD and GSH levels, accompanied by inhibiting expression level of 4-HNE. Likewise, GFG decreased APAP-induced the expression of cytochrome P450 E1 (CYP2E1). Pre-treatment with GFG significantly inhibited pro-inflammatory factors tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), Bax, Bcl-2 and cyclooxygenase-2 (COX-2) levels expression of which contributed to ameliorating APAP caused hepatotoxicity. Furthermore, liver histopathological observation provided further evidence that GFG pretreatment significantly inhibited APAP-induced hepatocyte necrosis, inflammatory cell infiltration. **Conclusion:** The present study clearly showed that GFG exerted a protective effect against APAP-induced hepatotoxicity due to its anti-oxidant, anti-apoptotic and anti-inflammatory effects.

**Key words:** Ginsenosides, *Panax ginseng*, acetaminophen, hepatotoxicity, anti-inflammation, anti-apoptosis

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Acetaminophen (APAP) is the most commonly drug that used for antipyretic analgesic and anti-inflammation[1]. Although APAP is a generally safe and effective at normal therapeutic doses, an excessively high dose of APAP may cause serious liver damage and death[2]. An overdose of APAP saturates the glucuronidation and sulfation pathways, causing massive accumulation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes glutathione (GSH) [3], covalently binds to cellular proteins and catalyzed by cytochrome P450 enzymes, such as CYP2E1[4]. Therefore, NAPQI triggers initiation of the injury process, ultimately leading to hepatocellular necrosis[5]. Oxidative/nitrosative stress is another important mechanism postulated as an important factor in the development of APAP-induced liver injury[6]. Treatment with APAP increases the production of reactive oxygen/nitrogen species (ROS and RNS), ATP depletion, hepatocyte injury and subsequent liver cellular necrosis[7]. Therefore, it would be of great value to develop more effective and safe drugs for prevention and therapeutic intervention strategies to prevent APAP-induced hepatotoxicity.

Ginseng (*Panax ginseng* C. A. Meyer), one of the famous Chinese herbal medicines, is widely used in China, Canada and United States for more than 2,000 years[8]. Similar to roots, the fruits of *P. ginseng* as non-medical part, have various chemical constituents (especially ginsenosides) including Re, Rb1, Rb2, Rc, Rg1, Rg2 and Rd[9-12]. Recent studies showed that ginsenosides from the fruits of *P. ginseng* (GFG) exert anti-cancer activity *in vitro*[13]. However, the ameliorative effect of GFG against APAP-induced liver injury in mice has not been investigated and such work could be useful and meaningful to gain a new understanding on the medicinal properties. Therefore, the aim of the study was to investigate potential effects of GFG in preventing acute hepatic injury triggered by APAP in mice.

## MATERIALS AND METHODS

**Chemicals and reagents:** The main ginsenoside in GFG was identified with Re of 20%, Rc of 3.14%, Rb2 of 3.15%, Rd of 8.04%. The APAP was purchased from Sigma-Aldrich (St. Louis, MO). The commercial assay kits for alanine aminotransferase (ALT), aspartate transaminase (AST), reduced glutathione (GSH), malondialdehyde (MDA) and hematoxylin and eosin (H and E) dye kits were purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). Rabbit monoclonal of Bax, Bcl-2, cyclooxygenase-2 (COX-2), cytochrome P450 E1 (CPY2E1), 4-hydroxynonenal (4-HNE) and secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). The Hoechst 33258 dye kit was obtained from Beyotime Biotechnology Co., Ltd. (Shanghai, China). The TUNEL apoptosis detection kit was provided with Roche Applied Science (Roche Applied Science, Germany, No. 11684817910). The ELISA kits of mouse TNF-α and IL-1β were purchased from R and D systems (Minneapolis, MN, USA). DyLight 488-labeled was provided by BOSTER Bio-Engineer Co., Ltd. (Wuhan, China). All the other chemicals were of the highest grade from commercial source.

**Animals and experimental protocol:** Eight-week-old male ICR mice (weighing 25~27 g) were provided by Experimental Animal Holding of Jilin University with Certificate of Quality No. of SCXK (JI) 2016-0003 (Changchun, China). The animals were housed in a laminar air-flow room maintained at a temperature of 23~27°C and humidity 50~70% throughout the study. All mice used in this study were continuously fed standard chow pellet diet, drinking water *ad libitum* and were maintained on a 12 h light/dark cycle. All experimental procedures were approved by Ethical Committee of Jilin Agricultural University (Permit Number: ECLA-JLAU-16063).

After acclimation for one week, the ICR mice were randomly divided into 4 groups as follows: Normal group, APAP-treated group and GFG-treated groups (150 and 300 mg kg$^{-1}$, respectively). The GFG powder was suspended in 0.05% carboxymethylcellulose sodium (CMC-Na). The GFG was administered by intragastrically administration. The normal group and the model group were given the same amount of 0.9% saline every day for 7 days. On the 6th day, APAP group and pretreatment GFG groups intraperitoneal injected a single of APAP (250 mg kg$^{-1}$, dissolved in warm saline) to induce hepatotoxicity in mice. Subsequently, at 24 h post-APAP mice were euthanized and sacrificed injection (Day 7). Blood were collected and then centrifuged and blood and livers were harvested. Blood was centrifuged to obtain plasma at 3000 g for 10 min and stored at -80°C for biochemical analysis. Meanwhile, the liver tissues were removed from the mice and weighed, the organ indices were calculated by the following formula:

$$\text{Organ indices (mg g}^{-1}) = \frac{\text{Organ weights}}{\text{Final body weights}}$$

A slice of liver tissues was kept in 10% neutral buffered formalin solution, embedded in paraffin and cut into 5 μm thick sections for histopathological staining and immunohistochemistry (IHC) analysis. For biochemical estimation, the remaining tissues were flash frozen in liquid nitrogen and stored at -80°C for further use.

**Measurement of biochemical marker:** The biochemical marker levels of AST, ALT, GSH and MDA activities were determined to evaluate APAP-induced liver injury with a commercial assay kits (Nanjing Jiancheng, China) according to the manufacturer's protocols.

**Determination of serum inflammatory markers TNF-α and IL-1β:** The levels of TNF-α and IL-1β in liver tissues were measured using commercial ELISA kits (R and D, Minneapolis, MN, USA) according to the manufacturer's instructions.

**H and E staining:** Samples were stained with H and E for the histopathological analysis and examined using a light microscope for histopathological examination (Leica, DN750, Solms, Germany). The histopathological characters were used for assessment of histological changes of the liver, including hepatocyte necrosis, inflammatory cell infiltration and congestion.

**Hoechst 33258 staining:** The sections were stained with Hoechst 33258 (10 µg mL$^{-1}$) and washed with PBS (phosphate buffer saline). The stained nuclei were visualized under UV excitation and photographed under a fluorescent microscope (Olympus BX-60, Tokyo, Japan). Image-Pro Plus 6.0 software (Media Cybernetics, Maryland, USA) was applied to evaluate the degree of liver apoptosis.

**Immunohisto chemistry and immunofluorescence analysis:** For immunohisto chemical analysis, the tissue samples were deparaffinized and rehydrated, then treated with citrate buffer solution (0.01 M, pH 6.0). After washed with Tris-buffered saline (TBS 0.01 M, pH 7.4), the sections were incubated with 1% bovine serum albumin (BAS) for 30 min at 37 temperature. Then, the liver sections were incubated with primary antibodies including COX-2 (1:200), Bax (1:200) followed by secondary antibody goat anti-rabbit IgG. Then, sections were exposed to diaminobenzidine (DAB) staining and hematoxylin counter-staining. The immunostaining intensity was analyzed by light microscopy (Olympus BX-60, Tokyo, Japan).

In brief, immunofluorescence was carried out as described for immunohistochemistry. The sections were incubated overnight at 4 with the rabbit anti-mouse CYP2E1

antibody (1:200), 4-HNE antibody (1:100). Then, the liver tissues were exposed to the DyLight 488-labeled secondary antibody (BOSTER, Wuhan, China). Nuclear staining was stained using 4'-6diamidino-2-phenylindole (DAPI). Immunofluorescence staining was visualized using a Leica microscope (Leica TCS SP8, Solms, Germany). Images were captured by a Leica microscope (Leica TCS SP8, Solms, Germany).

**Western blot analysis:** Western blot analysis was performed to measure the total protein levels of Bax and Bcl-2 in the mice kidney. The proteins were separated with 12% SDS polyacrylamide gels and transferred to a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked in 5% non-fat milk and incubated with primary antibodies against Bax (1:200) and Bcl-2 (1:200). Then, the membranes were incubated with the secondary antibodies. The intensity of the bands was assayed by computer Image plus 6.0 software (Media Cybernetics, Rockville, MD, USA).

**Statistical analysis:** Data were presented as the Mean±SD and analyzed using SPSS 17.0 software (SPSS, Chicago, IL, USA) for the least significance difference (LDS) multiple-comparison tests. Significant of difference was determined among the groups using one-way analysis of variance (ANOVA) followed by Tukey-Kramer tests. The p<0.05 or p<0.01 were considered statistically significant. Statistical graphs were presented by GraphPad Prism 6.0.4. (GraphPad Software, La Jolla, CA, USA).

## RESULTS

**Effect of GFG on APAP-induced deteriorative organ indices in mice:** Organ indices of the liver and spleen were considered as a putative indicator of health. As described in Table 1, in the present study, the organ indices of the liver and spleen were assessed in mice after exposed to APAP. Liver and spleen indices increased dramatically after 24 h APAP injection (p<0.05) compared with normal group, whereas, decreased in the 150 and 300 mg kg$^{-1}$ GFG groups (p<0.05). These results showed that GFG attenuated APAP induced hepatic injury by up-regulating organ indicator in mice.

Table 1: Effects of GFG on organ indices and serum biochemical markers in mice

| Groups | Dosage (mg kg$^{-1}$) | Organ indices (mg g$^{-1}$, ×100) | | Transaminases (U L$^{-1}$) | |
|---|---|---|---|---|---|
| | | Liver | Spleen | ALT | AST |
| Normal | - | 5.6±0.43 | 0.40±0.02 | 10.92±4.41 | 25.75±9.89 |
| APAP | - | 6.0±0.53* | 0.53±0.02* | 101.02±8.23** | 45.94±14.36** |
| APAP+GFG | 150 | 5.3±0.44# | 0.46±0.02# | 62.45±16.13## | 27.57±4.22# |
| APAP+GFG | 300 | 5.5±0.33# | 0.41±0.01# | 14.05±3.62# | 25.83±9.56# |

Values represent the Mean±SD, n = 8, *p<0.05, **p<0.01 vs. normal group, #p<0.05, ##p<0.01 vs. APAP group, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase
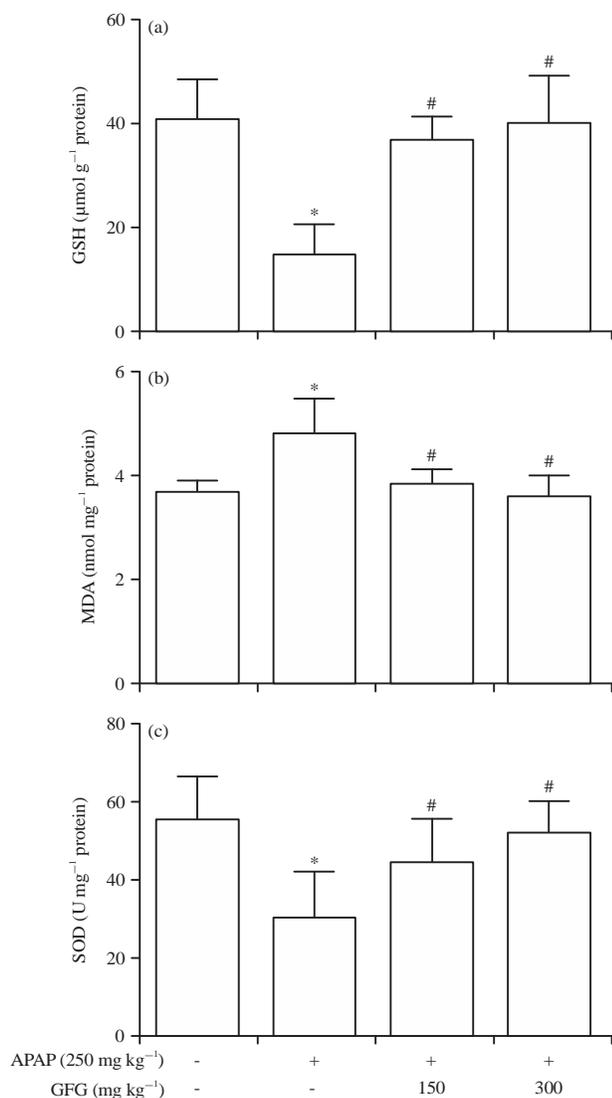
Fig. 1(a-c): Effects of GFG on the levels of (a) Glutathione (GSH), (b) Malondialdehyde (MDA) and (c) Superoxide dismutase (SOD) in APAP-induced hepatotoxicity

All data are expressed as Mean±SD, n = 8. *$p < 0.05$ vs. normal group, #$p < 0.05$ vs. APAP group

**Effect of GFG on APAP-induced liver dysfunction:** To evaluate whether there is protective effects of GFG against APAP-induced hepatotoxicity, the serum levels of ALT and AST were analyzed after APAP exposure. As performed in Table 1, the levels of two aminotransferase enzymes were both significantly increased following with a single APAP injection ($p < 0.01$), reflecting severe liver injury trigged by APAP administration after 24 h in mice. However, pre-treatment with GFG for consecutive 7 days (150 and 300 mg kg$^{-1}$) exerted a dose-dependent hepatoprotective effect compared to mice suffered from the APAP supplementation ($p < 0.05$ or $p < 0.01$).

These data demonstrated that GFG protected the liver from APAP induced aberrant increased transaminase in mice.

**Effect of GFG on APAP-induced oxidative stress:** Oxidative stress damage was involved in the mechanisms of APAP-induced hepatotoxicity. The GSH, MDA, SOD parameters were studied in APAP induced liver toxicity in mice. As shown in Fig. 1, APAP-treated significantly reduced the antioxidant parameters GSH and SOD contents and increased the MDA activity (14.82±5.9 µmol g$^{-1}$ protein, 57.31±6.9 U mg$^{-1}$ protein and 4.79±0.7 nmol g$^{-1}$ protein, respectively) ($p < 0.05$). In contrast, pre-treatment with GFG (150 and 300 mg kg$^{-1}$) for 7 days dramatically suppressed the over production of MDA content and restored the levels of GSH and SOD (3.85±0.2 nmol g$^{-1}$ protein and 3.61±0.4 nmol g$^{-1}$ protein, 36.93±7.4 µmol g$^{-1}$ protein and 39.77±10.4 µmol g$^{-1}$ protein, 47.41±6.1 U mg$^{-1}$ protein and 55.73±4.9 U mg$^{-1}$ protein) ($p < 0.05$). In brief, the results showed that GFG treatment may reduce the oxidative stress and restore endogenous antioxidant system to prevent APAP-induced hepatotoxicity.

In order to further verify, oxidative stress was related to the development of APAP-induced hepatotoxicity, immunofluorescence staining was performed to validate lipid peroxidation product 4-HNE on APAP-induced hepatotoxicity. As indicated in Fig. 2a, after APAP treatment for 24 h, strong 4-HNE fluorescence intensities were detected in hepatocyte near the central vena of the liver tissues. However, GFG pretreatment for 7 days dramatically reduced fluorescence intensities, especially in the high dose group. Moreover, the sites of lipid peroxidation were highly correlated with the necrotic regions in the liver. It was generally accepted that, APAP-induced hepatotoxicity is initiated by its metabolic activation, which is catalyzed by cytochrome P450 enzymes, mainly CYP2E1. Therefore, the expression of CYP2E1 in liver tissues after 24 h of APAP injection was evaluated. As expected, the expression of the CYP2E1 metabolizing enzyme was significantly increased when stimulated by APAP, whereas, GFG administration dose-dependently inhibited CYP2E1 activities (Fig. 2b). These results suggested that administration of GFG protected the liver from APAP-induced oxidative stress.

**Effects of GFG on inflammation of liver in APAP-treated mice:** The TNF-α and IL-1β are two important pro-inflammatory cytokines involved in the progression of APAP-induced hepatotoxicity. In the present study, the serum contents of TNF-α and IL-1β were determined by ELISA. As performed in Fig. 3, single APAP injection caused markedly higher levels of TNF-α and IL-1β (226.08±30.3 ng L$^{-1}$ protein and 2606.30±285.3 pg mg$^{-1}$ protein, respectively) than those

(a)          Normal                    APAP            APAP+GFG (150 mg kg*¹)   APAP+GFG (300 mg kg*¹)

H and E 1009
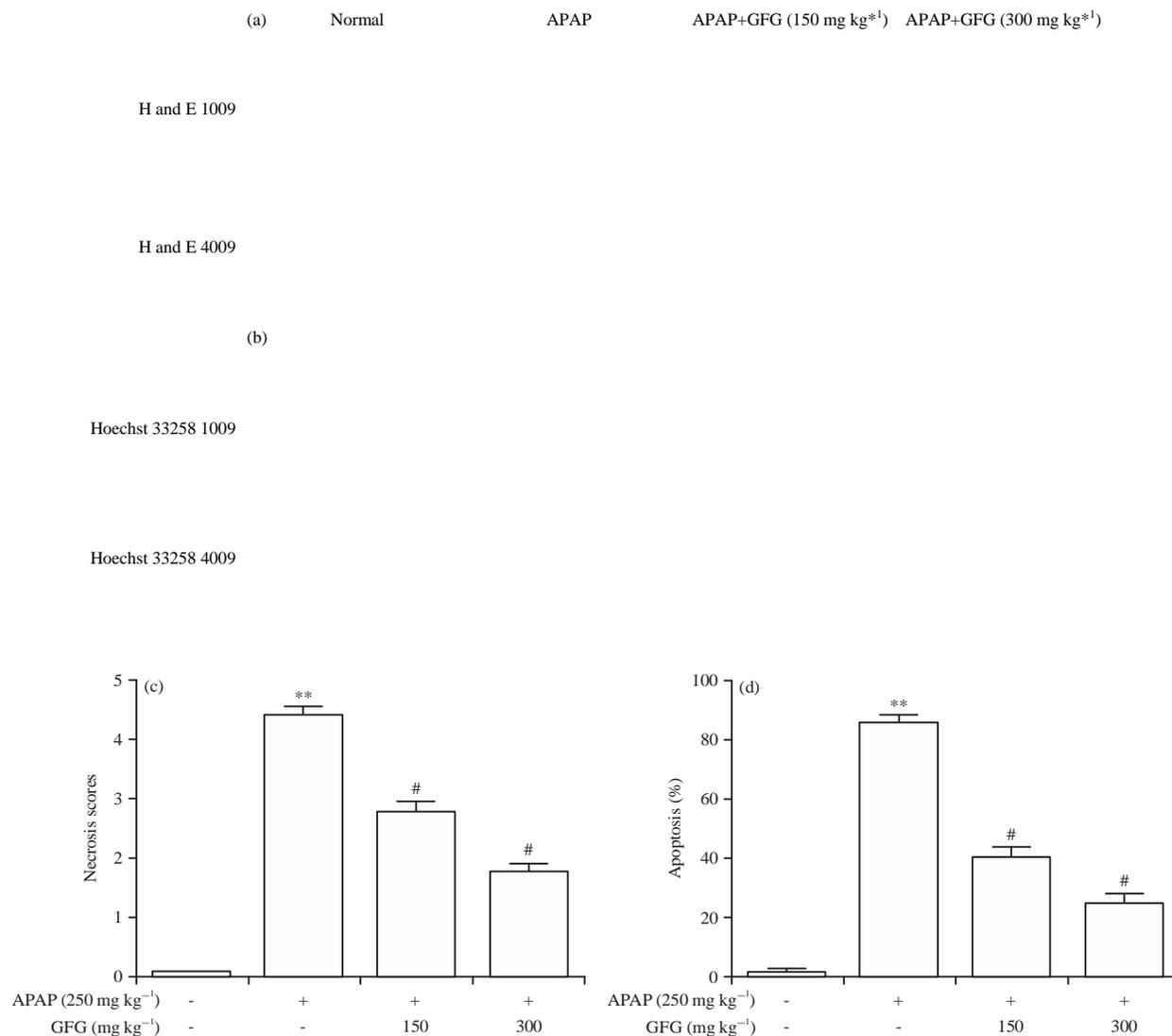
H and E 4009

(b)

Hoechst 33258 1009

Hoechst 33258 4009

Fig. 5(a-d): Histological examination of morphological changes in liver tissues. Liver tissues stained with (a) Hematoxylin-eosin
(H and E) (100×, 400×), (b) Hoechst 33258 (100×, 400×), (c) H and E necrosis scores, the percentage of apoptosis
(%) of (d) Hoechst 33258
All data were expressed as Mean±S.D., n = 8. **$p<0.01$ vs. normal group, #$p<0.05$ vs. APAP group

treated with GFG presented fewer COX-2 staining. These results implied that the protective effect of GFG might also attribute to its anti-inflammatory ability.

**GFG restores APAP-Induced liver histopathological changes:** Meanwhile, the protective effect of GFG was further confirmed by the histopathological evaluation of H and E staining. As performed in Fig. 5a, histology of liver tissue in the normal mice showed normal hepatic morphology with central-rounded well-defined vesicular nuclei and cytoplasm. Liver followed by APAP displayed sustained liver injury characterized by severe necrosis, apoptosis, inflammatory

infiltration and hydropic degeneration. By contrast, administration of GFG remarkably improved histopathological changes elicited by APAP at a dose-dependent manner. Furthermore, the histopathological changes in each section were accessed using necrosis scores, which represented the approximate necrotic extent around the central vein areas (Fig. 5c) ($p<0.05$ or $p<0.01$).

**Inhibitory effects of GFG on apoptosis:** In order to determine whether GFG treatment decreased liver cell apoptosis in APAP-induced acute liver injury, Hoechst 33258 staining was used to observe apoptosis of cell nucleus in the present study.
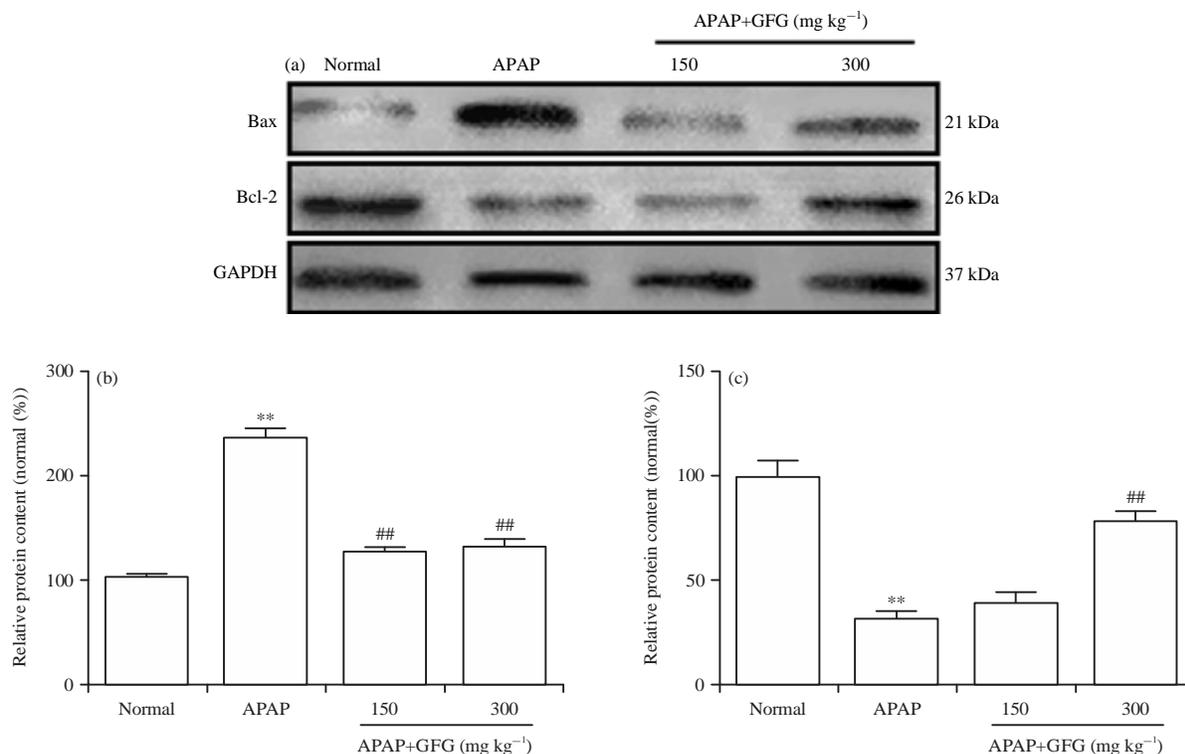
Fig. 6(a-d): Effects of GFG on the protein expression of: Bax, Bcl-2. Column chart show antibodies relative intensity. The protein expression was examined by western blot analysis in liver tissues from normal, APAP, APAP+GFG (150 mg kg$^{-1}$) and APAP+GFG (300 mg kg$^{-1}$)

All data were expressed as Mean±SD, n = 8. **$p<0.01$ vs. normal group, ##$p<0.01$ vs. APAP group

As shown in Fig. 5b and d, liver cell nucleus in APAP group were observed as significant nuclear fragmentation and condensation, indicating apoptosis of liver cells. However, after GFG pretreatment, most cell nucleus exhibited the round-shaped nuclei with homogeneous fluorescence intensity and regular contours. These findings showed that GFG exert potential anti-apoptotic properties against APAP-induced hepatotoxicity.

In order to further measure the extent of apoptosis in liver, the impacts of GFG on the pro-apoptotic factor Bax and anti-apoptotic factor Bcl-2 in all experimental groups were examined using immunohisto chemical and western blot analysis. As depicted in Fig. 4b and d, Bax positive expression was heterogeneous but was located in the cytoplasm of liver cells. The rate of positive expressions of Bax was found to be significantly lower in GFG pretreatment groups than in APAP group ($p<0.01$). The results of western blot showed that APAP exposure significantly increased the expression levels of Bax and decreased Bcl-2 compared with the normal group. But these changes could be effectively reversed with GFG pretreatment (Fig. 6) ($p<0.01$). Collectively, these results suggest that GFG may effectively block the APAP-induced acute liver injury by regulating the expression of Bcl-2 family.

## DISCUSSION

Current study clearly showed that GFG exerted a protective effect against APAP-induced hepatotoxicity, which may partly attribute to its anti-oxidant, anti-apoptotic and anti-inflammatory activities. In the present study, it was observed that AST and ALT levels were significantly increased in mice treated with 250 mg kg$^{-1}$ APAP, whereas, these increases could be weakened by GFG (150, 300 mg kg$^{-1}$). These results showed that GFG could relieve hepatic injury triggered by APAP exposure. Moreover, levels of MDA were significantly elevated while GSH and SOD were considerably decreased in the APAP-exposed mice. These results are in agreement with the results of Ding *et al.*[14], who indicated that oxidative stress plays a central role in the pathophysiology of APAP-evoked hepatotoxicity. However, GFG could alleviate oxidative stress and protected against APAP-induced liver damage. Additionally, H and E and Hoechst 33258 staining were used to validate that APAP-induced hepatic injury is characterized by liver cell necrosis, swelling, part of the inflammatory infiltration of cells, whereas, GFG treatment can reverse this structural change. These findings uncovered a concept that GFG impressively ameliorates APAP induced

intoxication through suppressing hepatocyte damage, which is dependent on activation of GFG. In addition, acute APAP injection triggered the levels of TNF-α and IL-1β are increased in the model group as characterized by loose and swollen of hepatocytes, the disappeared nucleus or necrotic. It is similar to previous study that IL-1β and TNF-α cause drug-induced hepatic injury[15]. From the immunofluorescence results, it is found that the positive expression of CYP2E1 and 4-HNE in GFG-treated groups were significantly reduced, suggesting that it could be related to the inhibition of GFG for oxidative stress and free radical scavenging. The immunohistochemistry and western blot analysis showed that Bax expression is dramatically elevated and the Bcl-2 expression is decreased in APAP administration group and relatively reversed by GFG administration, which is consistent with previous studies[16,17]. Meanwhile, GFG blocked the protein expression of COX-2, suggesting that GFG suppressed the inflammatory response in APAP mediated hepatotoxicity.

The APAP is safe and widely used at a normal dosage, the major elimination pathways of APAP are conjugation with glucuronide and sulfate[18]. However, unintentional overdosing is usually recognized after liver failure has developed in both humans and experimental animals[4,6,19] even death. Therefore, for the current study, GFG was used to assess the hepatoprotective activity by checking several biochemical markers, inflammatory indicators, oxidative and apoptotic relative proteins and histological changes in APAP-induced hepatotoxicity animal model.

In recent years, many active compounds extract from natural products attract more attention due to its lowed side effect and potential therapeutic value against APAP-induced acute liver injury[20-23]. Ginsenosides, prepared from the fruits of *Panax ginseng*, are popular chemical compound used for cancer[24], diabetes, autoimmune disease and Alzheimer's disease. However, the protective action of GFG against APAP caused hepatotoxicity has not been explored yet. Though the present work was similar to a previous report showing that ginsenoside Rg5 ameliorated acetaminophen-induced hepatotoxicity in mice with the same mouse model via inhibiting oxidative stress, inflammation and apoptosis[25], the different perspectives and meanings are shown in this study.

As a non-traditional medicinal part of ginseng, the utilization of the fruits is minimal, even are usually abandoned directly after the seeds are collected. Considering the constituents and potential medicinal value of the underutilized fruits, it we investigated the protective effect of GFG against APAP-induced liver injury and its potential molecular mechanisms. Compared with GFG, BG was generated from ginseng through steaming and drying, this wrought process needs time and energy. The total saponins from its fruits are abundant, such as ginsenosides Re and Rg1, which may have implications for the study of liver protection. These will be further studied in the future and may be used domestically for food applications. Additionally, recent study relevant to the effect of extract from GFG on plasma cholesterol level reduction and enteric neoplasm prevention in different experimental models were performed, which revealed the accumulating beneficial effects of GFG for multiple clinical researches[26].

As one of the final products of lipid peroxidation, the degree of expression of 4-HNE reflects oxidative damage in liver tissue. The CYP2E1, a member of the cytochrome P450 enzyme family, mainly metabolizes toxic electrophile NAPQI derived from APAP[27]. And CYP-mediated APAP bioactivation is the key initiating step of APAP hepatotoxicity.

Massive evidences revealed that acetaminophen induced translocation of concerning apoptosis in Bcl-2 family proteins[28], including the Bax, Bcl-2[29] and release of cytochrome c[30]. Moreover, COX-2 is believed to be the predominant cyclooxygenase involved in inflammatory responses.

The GFG effectively attenuated APAP-induced liver injury through various mechanisms. The molecular mechanism of hepatoprotective effects mediated by GFG administration against APAP liver injury might be partially due to anti-cell apoptosis, reduced inflammation and the inhibition of oxidative stress effects. Based on the present data, GFG produced therapeutic strategy to prevent drug induced APAP cytotoxicity. Taken together, GFG has considerable potential for development as a natural agent for the treatment of toxic acute liver failure.

## CONCLUSION

Current investigation is the first report indicating that pretreatment of GFG is has beneficial action in the prevention of APAP-induced hepatotoxicity by enhancing the cellular defense system and lowing inflammatory response in APAP model. It is concluded that the mechanism of hepatoprotective effects by GFG supplementation against APAP toxicity might be due to anti-cell apoptosis, reducing inflammation, inhibiting oxidative stress and finally preventing cell death and liver necrosis. Collectively, the present findings demonstrated that GFG may have safe therapeutic potential in protecting liver tissue from APAP toxicity. Importantly, these data also strongly emphasize that GFG seems to be an attractive supplementary drug candidate for the treatment and prevention of liver injury.

## SIGNIFICANCE STATEMENT

In this study, evaluated ginsenosides from the fruits of *Panax ginseng* attenuates liver injury in APAP induced mice by suppression of oxidative stress, inflammation, apoptosis and nitrative stress. It could be suggested that ginsenosides from the fruits can be used as an effect liver protection drug.

## ACKNOWLEDGMENTS

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