British Journal of Pharmacology and Toxicology 4(6): 256-261, 2013
DOI:10.19026/bjpt.4.5409
ISSN: 2044-2459; e-ISSN: 2044-2467

Submitted: August 24, 2013 Accepted: September 03, 2013 Published: December 25, 2013

Research Article

Protective Effect of Penta-acetyl Geniposide on Acute Liver Injury Induced By D-galactosamine in Mice

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Abstract: Penta-acetyl geniposide ($(Ac)_5GP$), an herbal derivative prepared from Gardenia Fructus geniposide, decreased the DNA damage and hepatocarcinogenesis induced by aflatoxin B_1 (AFB_1). The present study was carried out to further evaluate the protective effect of $(Ac)_5GP$ on liver injury induced by D-galactosamine (D-GalN). D-GalN (400 mg/kg) was given to mice by intraperitoneal injection, while $(Ac)_5GP$ were orally administered by gastric gavage. Spectrophotometrical method was used to measure activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) in serum and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA) in hepatic tissue. Hepatic histological structure was observed under light microscopy. The features of acute liver injury were observed in D-GalN-treated mice. $(Ac)_5GP$, similar to silymarin, decreased the elevated serum levels of ALT, AST and MDA and increased the reduced activities of SOD and GSH-Px induced by D-GalN. $(Ac)_5GP$ was also showed to be more effective than GP in reversing these abnormal changes and the levels of $(Ac)_5GP$ on MDA and SOD showed a dose-effect relationship. These biochemical observations were also supplemented by histopathological observations of the liver sections. It was concluded from the results that $(Ac)_5GP$ can produce protective effect on acute liver injury induced by D-GalN *via* an antioxidative mechanism. Therefore, $(Ac)_5GP$ may be developed as an efficient hepato-protective agent.

Keywords: Antioxidative effect, D-galactosamine, *gardenia*, liver injury, penta-acetyl geniposide

INTRODUCTION

Gardenia, the fruit of *Gardenia jasminoides Ellis*, has been widely used to treat liver and gall bladder disorders, such as hepatitis, acute jaundice and inflammation, in Chinese medicine for many years (Peng *et al*., 2004). Geniposide (GP), a main iridoid glycoside component isolated from *Gardenia Fructus*, has been shown to possess diverse pharmacological activities, such as anti-inflammatory (Liu *et al*., 2010; Yang *et al*., 2012), anti-oxidative (Liu *et al*., 2007, 2009; Ma *et al*., 2011), anti-tumor (Cao *et al*., 2010; Peng *et al*., 2005), anti-diabetic (Kojima *et al*., 2011; Wu *et al*., 2009), anti-angiogenic activities (Koo *et al*., 2004), *et al*. Since it is difficult to isolate glycoside due to its high polarity, penta-acetyl geniposide ($(Ac)_5$ GP), an acetylation derivative, was developed in 100 g scale to simplify the isolation process. The structure was identified by mass spectrophotometry and nuclear magnetic resonance. In comparison with $(Ac)_5GP$ and GP, $(Ac)_5GP$ exhibited the ability to activate GST and GSH-Px as GP in AFB_1-treated hepatocytes and induced the activation of GST earlier than the GP-treated group. $(Ac)_5GP$ was shown to be more effective in inhibiting AFB_1-induced unscheduled DNA synthesis in the concentration range 0.02-0.1 mM (Tseng *et al*., 1994).

In addition, $(Ac)_5GP$ can inhibit AFB_1-induced hepatic lesions, preneoplastic changes and gamma glutamyl transpeptidase (γ-GT)-positive foci development. Although no differences were found in total number of γ-GT foci, the treatment of $(Ac)_5GP$ significantly reduced the number of AFB_1-induced γ-GT-positive foci with diameter larger than 0.3 mm (Lin *et al*., 2000). $(Ac)_5GP$, as well as GP, could be pursued as chemopreventive agents, which anti-tumor effect and mechanism have further been investigated (Peng *et al*., 2005, 2006).

Since $(Ac)_5$ GP has a protective effect on AFB_1-induced DNA damage in hepatocytes and hepatotoxicity in rats, it is interesting to further know the preventive effect of $(Ac)_5GP$ on D-GalN-induced acute liver injury in mice. In this study, $(Ac)_5GP$, similar to silymarin, decreased the elevated serum levels of ALT, AST and MDA and increased the reduced activities of SOD and GSH-Px in D-GalN-treated mice. These results showed a protective effect of $(Ac)_5GP$ on D-GalN-induced acute liver injury in mice.

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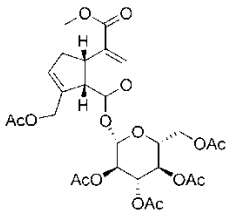


Fig. 1: Chemical structure of penta-acetyl geniposide

MATERIALS AND METHODS

Materials: GP (purity>80% by HPLC, provided by Hefei XinFeng Co. Ltd, P. R. China) was added to a mixed solvent of pyridine and acetic anhydride (2:1), the mixed solution was stirred overnight at room temperature. The reacting product was extracted with ethyl acetate. The organic layer was washed with water, dried with anhydrous sodium sulfate, filtrated and concentrated *in vacuo*. The product was obtained by recrystalization with ethanol as a white powder (purity>98% by HPLC). M.p. 116-117°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.98-2.09 (5×3H, s), 2.17 (1H, m), 2.85 (2H, m), 3.21 (1H, m), 3.73 (4H, m), 4.16 (1H, dd, *J* = 2.3, 12.4 Hz), 4.25 (1H, dd, *J* = 4.4, 12.4 Hz), 4.70 (2H, m), 4.87 (1H, d, *J* = 8.1 Hz), 5.01 (1H, dd, *J* = 8.1, 9.5 Hz), 5.09 (1H, d, *J* = 6.0 Hz), 5.11 (1 H, d, *J* = 9.6 Hz), 5.23 (1H, d, *J* = 9.5 Hz), 5.84 (1H, s), 7.42 (1H, d, *J* = 0.8 Hz); TOF-MS *m/z*: 621.1789 (Calcd for $C_{27}H_{34}NaO_{15}$: 621.1790) (Fig. 1) (Lin *et al*., 2000).

Animals and experimental design: Six-eight-week old male Kunming mice, 20-30 g, were obtained from the Animal Department of Anhui Medical University and maintained in a controlled temperature at 23±2°C and 50±10% relatively humidity with a 12 h light/dark cycle and acclimatized for at least one week prior to use. Animals were housed in plastic cages with free access to food and water. All animals received human care in compliance with the Guidelines of the Animal Care and Use of Laboratory Animals as set by the Association of Laboratory Animal Sciences at Anhui Medical University.

Mice were randomly divided into seven groups with 10 mice per group: normal control group, model group (D-GalN group), $(Ac)_5$GP (50, 100, 150 mg/kg) groups, the silymarin group and GP group (Fu *et al*., 2007). Mice were administered orally by gastric gavage with different doses of $(Ac)_5$GP or silymarin or GP at a volume of 20 mL/kg once a day for 10 days; the normal control group, D-GalN group, GP group and the silymarin group were administered with an equivalent volume of 0.3% sodium carboxymethyl cellulose saline to dissolve GP and $(Ac)_5$GP in water. On day 10, at 2 h after the final administered of $(Ac)_5$GP, GP or silymarin, the mice were intraperitoneally injected with D-GalN at the dose of 400 mg/kg as a physiological saline and the mice in the normal control group were intraperitoneally injected with an equivalent volume of physiological saline alone. At 16 h after the D-GalN injection, each mouse was weighed and retro-orbital bleeding to execute for blood collection, then killed by cervical dislocation. Serum was obtained from the collected blood by centrifugation immediately for measuring activities of AST and ALT and livers were weighed to calculate the liver index (liver index = liver weight/body weight of mice) and take the same parts of liver 0.5 g, to pre-cooling 0.9% saline made of 10% liver homogenates for measuring activities of SOD, GSH-Px and MDA content levels.

Data collected:

Biochemical determination: Serum levels of ALT and AST, liver homogenate SOD, GSH-Px and MDA were determined using commercial analysis kits obtained from the Jiancheng Institute of Biotechnology (Nanjing, China).

Histopathological observation: Few millimeter-thick midsection of the left lobe of liver from each animal was processed for observation by light microscopy. The process involved fixing the tissue specimen in 10% neutral buffered formalin solution, preparing the block in paraffin, cutting into 5-6 μm thick sections and staining the sections with haematoxylin-eosin stain. Stained sections were observed under light microscopy (Olympus BX50; Olympus Japan) and later subjected to image analysis (BI 2000; TaiMeng Technology, China). The percentage area of necrosis was determined by dividing the area of necrosis by the sum of the reference area of ten low power fields.

Statistical analysis: Results were represented as means±S.D. and all statistical comparisons were made by one-way ANOVA test followed by Tukey post-hoc analysis and *p*-values less than or equal to 0.05 were considered to be statistically significant.

RESULTS

Effect of $(Ac)_5$ GP on liver indices and serum levels of ALT and AST: The relative liver weights were significantly augmented after treatment with D-GalN alone compared to normal control group. In contrast, pretreatment with $(Ac)_5$GP (50, 100, 150 mg/kg) and silymarin (150 mg/kg) significantly reduced the relative liver weights compared to model group that received D-GalN alone, however, pretreatment with GP (100 mg/kg) had no effect on the relative liver weights.

Table 1 showed that the injection of D-GalN causes a significant elevation of serum ALT and AST levels in mice. The serum enzymes (AST and ALT), the soluble enzyme within the cytoplasm of hepatocytes, are the markers of D-GalN-induced liver damage, when liver cell was injured, it increased permeability of cell membranes, resulting in ALT and AST release into the

Table 1: Effect of $(Ac)_5$GP on liver index (%), ALT and AST activities in serum of mice

Group	Dose (mg/kg)	Liver index (%)	ALT (U/L)	AST (U/L)
Normal	-	3.89±0.55	11.71±0.87	14.62±1.78
Model	-	5.35±0.30$^{\#\#}$	25.14±2.01$^{\#\#}$	27.68±2.79$^{\#\#}$
GP	100	4.98±0.08	21.90±1.92**	20.23±3.21**
silymarin	150	3.76±0.13**	17.63±1.79**	19.12±1.83**
$(Ac)_5$GP	50	4.27±0.21**	14.85±1.38**	18.29±2.51**
$(Ac)_5$GP	100	4.08±0.37**	13.87±1.42**	17.65±1.87**
$(Ac)_5$GP	150	3.88±0.21**	12.12±0.98**	16.23±1.93**

Data were represented as means±S.D. (n = 10 in each group) from seven separate experiment. $^{\#\#}$p<0.01 vs normal group. **p<0.01 vs model group

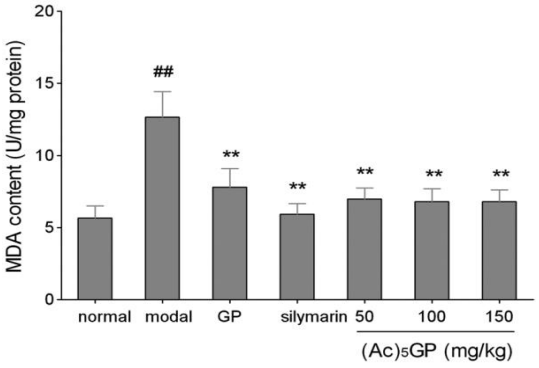
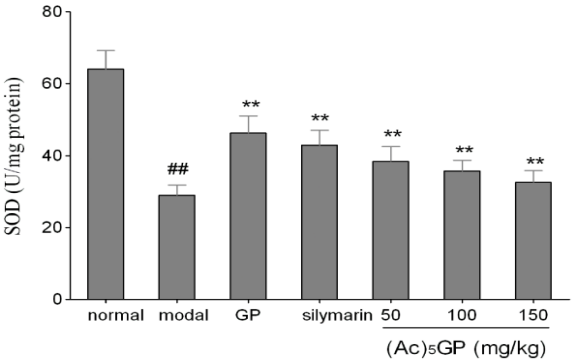


Fig. 2: Effect of $(Ac)_5$ GP on MDA content in liver of mice treated with D-GalN: Data were represented as means±S.D. (n = 10 in each group) from seven separate experiment; $^{\#\#}$p<0.01 vs normal group; **p<0.01 vs model group

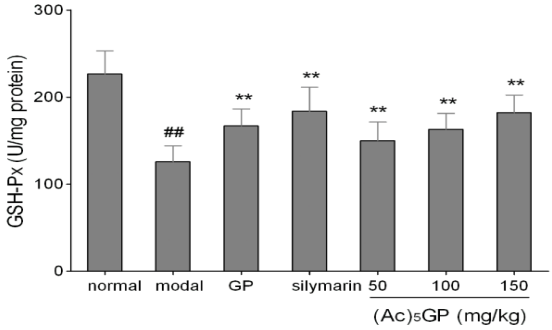
blood, the serum levels of ALT and AST can reflect the degree of liver cell injury. Pretreatment with $(Ac)_5$GP (50, 100, 150 mg/kg), GP (100 mg/kg) and silymarin (150 mg/kg) attenuated the D-GalN-induced increase in ALT and AST levels ($p<0.05$). $(Ac)_5$GP (50, 100, 150 mg/kg) reversed the above changes and the levels of serum ALT and AST showed a dose-effect relationship. The serum levels of ALT and AST were decreased in silymarin-treated group (150 mg/kg) ($p<0.05$), while the decrease of serum level in $(Ac)_5$GP-treated groups was more significant (Table 1).

Effect of $(Ac)_5$GP on hepatic lipid peroxidation: Lipid peroxides and its metabolite MDA can damage the cell membrane structure, leading to cell swelling and necrosis, MDA content reflects the degree of peroxidative damage and cell damage. Figure 2 showed that D-GalN significantly increased MDA content in liver homogenates compared to normal control group. The abnormal change of this index in $(Ac)_5$GP (50, 100, 150 mg/kg) groups, the silymarin group and GP group was markedly ameliorated.

Effect of $(Ac)_5$GP on the levels of hepatic SOD and GSH-Px in mice: As shown in Fig. 3, antioxidant activities of $(Ac)_5$GP were estimated by examination of decrease in liver SOD and GSH-Px activity. The activities of control groups pretreatment with $(Ac)_5$GP hepatic SOD and GSH-Px activity. The activities of model group treated with D-GalN alone caused a



(a)



(b)

Fig. 3: Effect of $(Ac)_5$ GP on the levels of the liver SOD and GSH-Px in mice Data were expressed means±S.D. from seven separate experiment; $^{\#\#}$p<0.01 vs normal group; **p<0.01 vs model group

(50, 100, 150 mg/kg), GP (100 mg/kg) and silymarin (150 mg/kg) markedly increased the levels of SOD and GSH-Px.

Liver histopathology: Liver tissues were collected to assess the effect of $(Ac)_5$GP on liver pathological changes. No histological abnormalities were observed in normal control mice. The hepatic parenchyma appeared normal and hepatocytes were arranged around the central vein (Fig. 4a). The livers of mice treated with D-GalN alone showed marked centrilobular necrosis in hepatocytes, with marked mononuclear cell infiltration (Fig. 4b). Silymarin group obviously improved the degree of damage and liver cell basic

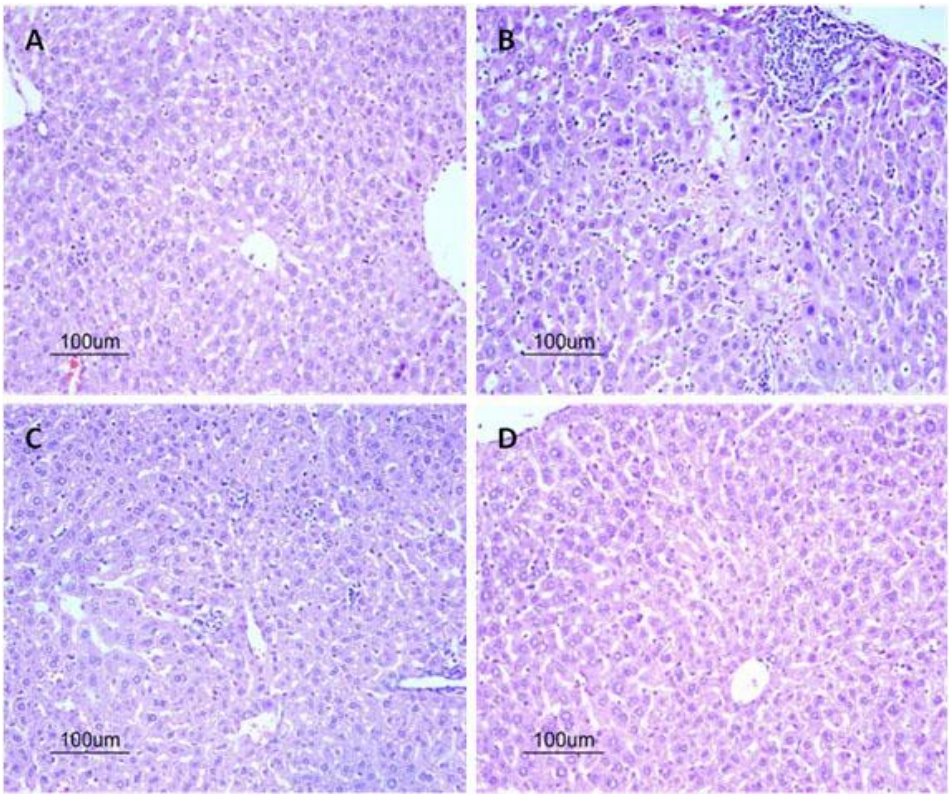


Fig. 4: Effect of $(Ac)_5$ GP on the histological structure of liver: Histopathological examinations performed under a light microscope (magnification×100) on liver specimens obtained 16 h after D-GalN treatment. Figure 3A to D represent typical histological changes from normal control group, model control group, 50 mg/kg $(Ac)_5$ GP group, 100 mg/kg GP group

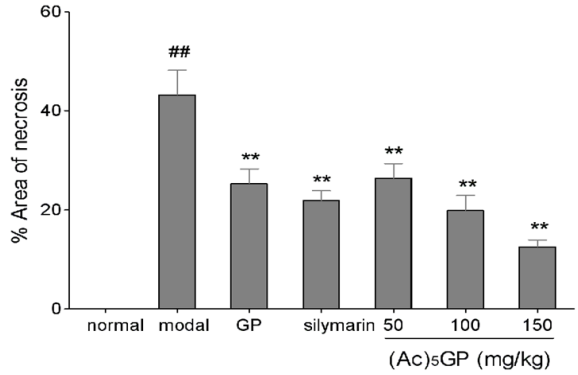


Fig. 5: The ratio of necrotic liver surface area by image analysis; Data are expressed as means±S.D; n = 10 per group; $^{\#\#}p<0.01$ vs normal group; $^{**}p<0.01$ vs model group

returned to the normal levels. The necrotic hepatocytes were characterized by cell enlargement and nuclear dissolution. The percentage area of necrosis was the highest in the D-GalN group. In the $(Ac)_5$GP (50 mg/kg) group, the degree of necrosis was less extensive than that in livers from mice with D-GalN alone (Fig. 4c and 5). A significant difference was also observed in the GP (100 mg/kg) (Fig. 4d). Hepatocyte necrosis induced by D-GalN was alleviated by pre-treatment of $(Ac)_5$GP. The changes of histopathological appearance and biochemical index from D-GalN-treated mice were significant compared to normal control group. The changes from $(Ac)_5$GP-pretreated mice showed significant hepato-protective effects of $(Ac)_5$GP against D-GalN-induced liver injury in mice.

DISCUSSION

D-GalN, an amino sugar selectively metabolized by hepatocytes, has been found to induce liver damage which closely resembles human viral hepatitis (Decker and Keppler, 1972). Oxygen-derived free radicals released from activated hepatic-macrophages are the primary cause of D-GalN-induced liver damage (Shiratori *et al*., 1988). And increased production of reactive oxygen species (ROS) has been observed in primary culture of rat hepatocytes damage induced by GalN (Quintero *et al*., 2002). The serum enzymes (AST and ALT) are the markers of D-GalN-induced liver damage. $(Ac)_5$GP (50, 100, 150 mg/kg) reversed these changes and the serum levels of ALT and AST showed a dose-effect relationship. These evidences suggested that the hepato-protective effects of $(Ac)_5$GP might be mediated through reducing ROS.

SOD is the antioxidant enzymes of the animal body, its can remove free radicals and prevent free radical damage to the cell structure, the size of its vitality reflects the antioxidant, free radical scavenging capacity. GSH-Px, along with SOD, is one of the body's endogenous antioxidants and is well-known to protect liver cells against oxidative damage through chemical or enzymatic reactions.

MDA, a secondary product of lipid peroxidation, is a useful indicator of tissue damage involving a series of chain reactions (Ohkawa *et al*., 1979). And a reduction in the activity of SOD is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes (Jayakumar *et al*., 2006). Reducing GSH constitutes the first line of defense against free radicals (Raja *et al*., 2006). GP and $(Ac)_5GP$ at all tested doses prevented elevation of liver MDA content, reduction of liver SOD activity and increase of GSH content resulted from mice liver intoxication with D-GalN. These observations further suggested that GP and $(Ac)_5GP$ had free radical scavenging activities.

Silymarin is used as standard hepatoprotective agent due to its excellent hepatoprotective activity in xenobiotic intoxication and fungal intoxication (Dixit *et al*., 2007). $(Ac)_5GP$, the same as silymarin, reversed the elevated serum levels of ALT, AST, MDA and the decrease of SOD and GSH-Px activity in D-GalN-treated mice. This supported that $(Ac)_5GP$ can be developed as a hepatoprotective agent.

Furthermore, compared to GP group (100 mg/kg), $(Ac)_5GP$ (100 mg/kg) was shown to more decrease the elevated serum levels of ALT, AST and MDA and more increase the reduced activity of SOD in D-GalN-treated mice. $(Ac)_5GP$ induced activation of GST earlier than the GP-treated group in AFB1-treated hepatocytes, which may faster inhibit cytochrome P450 monooxygenase (Tseng *et al*., 1994). These evidences indicated that $(Ac)_5GP$ may be a more potential hepato-protective agent than GP.

CONCLUSION

In conclusion, the present study reveals that $(Ac)_5GP$ shows a good hepato-protective effect on D-GalN-induced liver injury and this protection may be mediated through an antioxidative effect. $(Ac)_5GP$ may be more effective than GP in reducing D-GalN-induced hepatotoxicity in mice. From histopathological study, it also reveals that $(Ac)_5GP$ normalized the livers. *In vivo* evidence showed no toxic effects with $(Ac)_5GP$ and GP (Yamano *et al*., 1990). Therefore, this easily prepared $(Ac)_5GP$ can be developed as a more potential hepato-protective agent, which is necessary to further study the hepato-protective mechanisms.

ACKNOWLEDGMENT

The study was financially supported by National Natural Science Foundation of China (No. 20802003), TCM scientific research projects of Anhui Province Health Department 2012 (No. 2012zy46).

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