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Geniposidic acid protected against ANIT-induced hepatotoxity and acute intrahepatic cholestasis, due to Fxr-mediated regulation of Bsep and Mrp2

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ABSTRACT

Ethnopharmacological relevance: Geniposidic acid (GPA) is the main constituent of *Gardenia jasminoides Ellis (Rubiaceae)*, which has long been used to treat inflammation, jaundice and hepatic disorders. The cholagogic effect of *Gardenia jasminoides Ellis (Rubiaceae)* and GPA have been widely reported, but the underlying occurrence mechanism remains unclear.

Aim of the study: This investigation was designed to evaluate the hepatoprotection effect and potential mechanisms of GPA derived from *Gardenia jasminoides Ellis (Rubiaceae)* on fighting against α-naphthylisothiocyanate (ANIT) caused liver injury with acute intrahepatic cholestasis.

Materials and methods: Sprague-Dawley (SD) rats were intragastrically (i.g.) administered with the GPA (100, 50 and 25 mg/kg B.W. every 24 h) for seven consecutive days, and then they were treated with ANIT (i.g. 65 mg/kg once in the 5th day) which induced liver injury with acute intrahepatic cholestasis. Serum and bile biochemical analysis, bile flow rate and liver histopathology were measured to evaluate the protective effect of GPA fight against ANIT treatment. The protein and mRNA expression levels of farnesoid X receptor (Fxr), bile-salt export pump (Bsep), multidrug resistance associated protein2 (Mrp2), were evaluated to study the effect of liver protection about GPA against ANIT induced hepatotoxicity and underlying mechanisms.

Results: Some abnormalities were observed on ANIT treated rats including weight loss, reduced food intake and hair turned yellow. Obtained results demonstrated that at dose 100 and 50 mg/kg B.W. ($P<0.01$) and 25 mg/kg B.W. ($P<0.05$) of GPA pretreated dramatically prevented ANIT induced decreased in bile flow rate. Compared with ANIT treated group, the results of bile biochemical parameters about total bile acid (TBA) was increased by GPA at groups with any dose ($P<0.01$), glutathione (GSH) was increased significantly at high dose ($P<0.01$) and medium dose ($P<0.05$), total bilirubin (TB) was increased at high and medium dose ($P<0.05$), direct bilirubin (DB) was only increased at high dose ($P<0.01$). Serum levels of glutamic-Oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyltranspeptidase (γ-GT), TB, DB and TBA in comparison with ANIT treated group ($P<0.01$) were reduced by GPA (between 100 and 50 mg/kg B.W.) pretreatment. Histopathology of the liver tissue showed that pathological damages and hepatic portal area filled with bile were relieved after GPA pretreatment compared with ANIT treated group. The protein and mRNA expression of Fxr, Bsep and Mrp2 were decreased in ANIT treated group. On the contrary, the protein and mRNA of Fxr, Bsep and Mrp2 were up regulated significantly pretreatment by GPA at dose of high and medium groups. On protein level of Bsep and Mrp2 the result shown no statistical difference in GPA (25 mg/kg B.W.), but it was not same shown in mRNA level.

Conclusion: The results of this investigation have demonstrated that the GPA exerts a dose dependent hepatoprotection effect on ANIT induced liver damage with acute intrahepatic cholestasis in rats, which may due to Fxr mediated regulation of bile transporters like Bsep and Mrp2.

Abbreviations: GPA, Geniposidic acid; ANIT, α-naphthylisothiocyanate; Fxr, farnesoid X receptor; Bsep, bile salt export pump; Mrp2, multidrug resistance associated protein2; GOT, glutamic-Oxalacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GT, γ-glutamyltranspeptidase; TBA, total bile acid; TB, total bilirubin; DB, direct bilirubin; GSH, glutathione; UDA, ursodeoxycholic acid; RT-PCR, reverse transcription polymerase chain reaction; WB, western blotting

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1. Introduction

The liver as a large blood stream organ plays a vital role in the body, and it is also the main venue for drugs or toxicant to be metabolized. Thus, the liver is the highest risk organ to be exposed by toxic substances. Acute intrahepatic cholestasis is widely known as a common clinical pathological process in liver, can be caused changes such as intrahepatic bile duct stenosis, occlusion, bile acid secretion pattern change that ultimately cause hepatobiliary injury (Hirschfield et al., 2013). As we know, many factors can cause intrahepatic cholestasis like viral hepatitis (Belay et al., 2015) or autoimmune hepatitis (Wang et al., 2015), drug induced hepatic injury (Oh et al., 2015; Pillukat et al., 2014) and non-alcoholic liver disease (Virukalpattigopalratnam et al., 2013; Canet and Cherrington, 2014), primary biliary cirrhosis (Wang et al., 2015), cholestasis of pregnancy (Stieger, 2011), metastatic liver cancer (Okano et al., 2014) and other hereditary diseases (Więcek et al., 2015). The purpose of acute intrahepatic cholestasis therapy is to reverse injury from bile duct inflammation, forestall disease progression, and prevent the development into chronic cholestasis or other cholestatic liver disorders. Currently, drugs can be used to treat cholestasis only including Corticosteroids, Budesonide, Fibrates, Ursodeoxycholic acid (Purohit and Cappell, 2015), but its treatment efficacy was still not completely satisfied (Pou pon, 2012). Therefore, cholagogue antiphlogistic drug need to be found and verified imminently from the rich resources of Chinese herbal medicine.

Alfa-naphthylisothiocyanate (ANIT) was extremely common used in rodents as an inductive agent to build rat acute intrahepatic cholestasis models for research (Cui et al., 2009). ANIT is a typical compound currently which is best known to cause cholangiolitic hepatitis characterized by bile duct obstruction, serious interlobular duct epithelial apoptosis or necrosis, neutrophil infiltration around bile ducts (Pratima et al., 2006). The pathological changes of intrahepatic cholestasis caused by ANIT were bile duct epithelial cell damages and deceleration of bile flow, and the changes were dose dependent with highly spontaneous recovery (Ohta et al., 2007).

Fxr (Makishima et al., 1999), also known as a bile acid receptor) mediated bile acid transport and metabolism in the hepatocyte, and it was the first nuclear receptor identified to have bile acids as endogenous and physiologically relevant ligands (Owsley and Chiang, 2003). Fxr plays important role in respect of key enzymes and target genes of bile acid metabolism (Zollner et al., 2010), and also can regulate the synthesis of bile acids and maintain homeostasis and avoid liver injury. Current studies found that knock out functional gene of Bsep (Stieger and Geier, 2011) and Mrp2 (Zweers et al., 2012) or inhibit their expression and reduce their sites in the distribution of rats, the cholestasis occured, it showed that Bsep and Mrp2 expression or dysfunction was closely related to the onset of cholestasis. Toxic bile acids such as conjugates of lithocholic acid could also be eliminated by the canalicular ABC-transporter as known as multidrug resistance protein. Thus, up-regulated the expression or recovered the function of Bsep and Mrp2 (Shoda et al., 2004) was one of key method for cholestasis treated.

Gardenia jasminoides Ellis (Rubiaceae) is the fruit of the gardenia plant (*Gardenia jasminoides Ellis*), which is a traditional Chinese medicine that has long been used to treat febrile diseases, jaundice hepatitis (Lee et al., 2006), torsion contusion (Chen et al., 2014), hypertension (Higashino et al., 2014), diabetes (Kojima et al., 2011) and pyogenic infections. Iridoid glycosides (Yu et al., 2012) are the most major components of *Gardenia jasminoides Ellis (Rubiaceae)*. Genipin was isolated from *Gardenia jasminoides Ellis* that offer marked hepatoprotection against damage induced by GalN/LPS related with its antioxidative and anti-apoptotic activities (Kim et al., 2010). According to reports, iridoid glycosides can up-regulate the bile efflux transporter to increase bile excretion, reduce liver toxicity that cause by bile duct epithelial apoptosis or necrosis, neutrophil infiltration around bile ducts (Harada et al., 1974). Geniposidic acid (GPA) (Fig. 1) ((1S, 4aS, 7aS)-1-(b-D-glucopyranosyloxy)-7-(hydroxylmethyl)-1, 4a, 5, 7a-tetrahydrocyclopent

-a[c]pyran-4-carboxylicacid) is one of the main iridoid glucoside compounds of gardenia fruit (Zhang et al., 2013), which has been regarded as a hepatoprotective component in rat hepatocytes. Therefore GPA may become an important active ingredient to be used for hepatoprotective drugs, and its hepatoprotective can be studied and designed according to this review article (Block et al., 2015), and it also might provide novel therapeutic intervention.

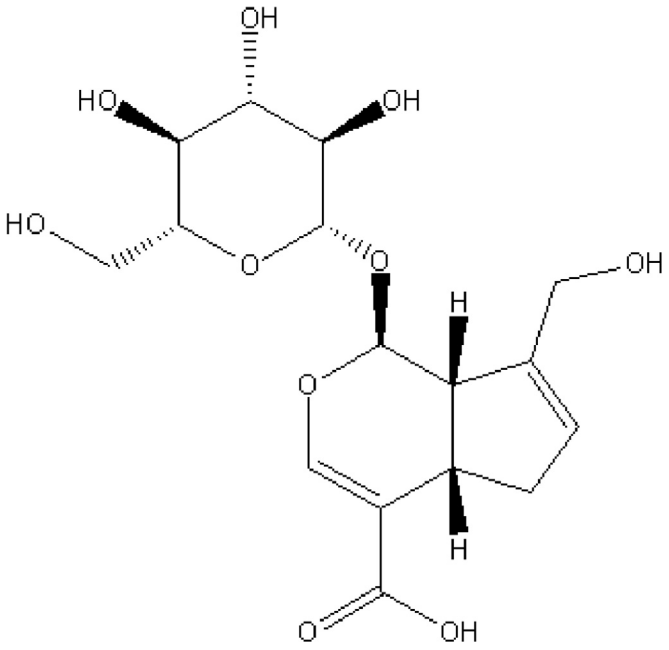

Fig. 1. Structures of geniposidic acid ($C_{16}H_{22}O_{10}$:374.34).

Thus, in this study, it is aimed to explore the hepatoprotective and choleretic effects of GPA, which can improve symptoms of ANIT induced intrahepatic cholestasis in rat. Reverse transcription polymerase chain reaction and western blot were used for further to clarify the potential mechanisms ultimately.

2. Materials and methods

2.1. Drugs and Main Reagents

GPA (Lot number: 20140511039) with the purity more than 98% (Fig. 2.) was obtained from Nanjing Zelang pharmaceutical technology co., LTD (Nanjing, China). The extraction procedure was as follows (Kim et al., 2013). 10 kg (kg) dried fruits of gardenia jasminoides were crushed into small pieces and filtered through a 20-mesh (0.85-mm) sieve, and then the pieces were soaked into 40 L (L) methanol at room temperature for 24 h. The methanol extract was concentrated and evaporated to dryness under reduced pressure, and the residue (3.95 kg) was dissolved or suspended in water (4 L), and then the suspension was serially partitioned with petroleum ether, ethyl acetate, n-butanol to obatain petroleum ether (726.8 g), ethyl acetate (117.6 g), n-butanol (646.7 g), and H_2O (1.23 kg) parts, and n-butanol part was fractionated on silica gel column through gradient elution with Ethyl acetate–Methanol–H_2O (90:5:5, 80:13:7, 70:20:10, and 60:25:15).