Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association 80 (2017) 623–629

www.jcma-online.com

Original Article

Hepatoprotective effects of ethanol extracts from *Folium Syringae* against acetaminophen-induced hepatotoxicity *in vitro* and *in vivo*

Chen-Xi Shi [a], Yue-Xia Lin [a], Fang-Ping Liu [a,*], Yi-Cong Chang [a], Rui Li [a], Chang-Wen Li [b], Ying Li [a], Jing-Shan He [a], Xin Ma [a], Zhi Li [a]

[a] *Basic Veterinary Department, College of Veterinary Medicine, Northeast Agricultural University, Harbin, China*
[b] *Laboratory Animal Base, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences, Harbin, China*

Received December 22, 2016; accepted March 30, 2017

Abstract

Background: The leaves of *Folium Syringae* (FS) have been long used as a traditional Chinese folk medicine for their anti-inflammatory effect, utilized as an antibacterial and antiviral treatment. The purpose of this study was to investigate the potential hepatoprotective effects of FS on acetaminophen-induced hepatic injury in primary hepatocytes and mice.

Methods: Hepatocytes obtained by the inverse perfusion method were divided randomly into five groups. Prior to acetaminophen exposure, 3 different doses of FS ethanol extracts were given to hepatocytes and mice, respectively. Thereafter, transaminases, glutathione S-transferase A1 (GSTA1) and some hepatic indices were determined.

Results: FS ethanol extracts (200 μg/mL) pretreatment prevented all of the alterations, returning their levels to nearly those levels observed in the control group *in vitro*. Treatment with FS ethanol extracts (200 mg/kg) significantly reduced the toxicity induced by acetaminophen *in vivo*, which manifested as a decrease in transaminases, and the hepatoprotective effects of FS were similar to Silymarin (positive group). GSTA1 represented the same change trend as transaminases and hepatic indices, and at a dose of 100 μg/mL FS ethanol extracts *in vitro* and 100 mg/kg *in vivo*, GSTA1 content changed significantly ($p < 0.01$), but transaminases were insignificant ($p > 0.05$).

Conclusion: The results of our investigation suggested that FS ethanol extracts possess significant protective effects against hepatotoxicity induced by acetaminophen both *in vitro* and *in vivo*. In addition, GSTA1 could be used as an indicator assessing the extents of hepatic injury, which is more sensitive than transaminases.

Keywords: Acetaminophen; *Folium Syringae*; Glutathione S-transferase A1; Hepatic injury

1. Introduction

Folium Syringae (FS), the leaves of *Syringa vulgaris* Linn., *Syringa dilatata* Nakai. and *Syringa oblata* Lindl., have all been used as a medicine for antibacterial and antiviral treatment in folk medicine in China. The main compositions of FS include organic acids, eugenol, glycosides and volatile oil, which have many pharmacological actions including free radical scavenging, antioxidation, antibacterial and antiviral activity, and anti-inflammatory and hypoglycemic function in diabetes. These effects have been widely researched, but there are few reports concerning the hepatoprotective effects. It has been reported that eugenol could stop the spread of free radicals[1] and protect the cell membrane lipid from peroxidation by inhibiting the formation of free radicals.[2] Since free radicals are related to liver injury, further inquiry and development regarding the hepatoprotective effects of FS is of great significance.

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Fang-Ping Liu, College of Veterinary Medicine, Northeast Agriculture University, 59, Mucai Street, Harbin 150030, Heilongjiang, China.

E-mail address: fangpingliu@126.com (F.-P. Liu).

http://dx.doi.org/10.1016/j.jcma.2017.03.007

Acetaminophen (APAP) is widely used as a kind of analgesic-antipyretic, but an excessive dose may cause hepatic injury.[3] The hepatotoxicity induced by APAP has been deemed to be caused by N-acetyl-para-benzoquinoneimine (NAPQI), a cytochrome P-450-mediated intermediate metabolite which leads to the depletion of glutathione (GSH).[4] GSH depletion caused by NAPQI causes oxidative stress and finally results in cell death.

Glutathione S-transferases (GSTs) are a family of phase Ⅱ drug-metabolizing enzymes, and the main GST of the human liver is Alpha class (GSTA),[5] in which GSTA1 plays a key role in protecting against toxic electrophiles and products of oxidative stress because of its active sensibility to lipid peroxide and its derivations.[6] GSTA1, mainly through numerous xenobiotics such as carcinogens, environmental toxins, some drugs combined with GSH, promote the degradation of clearance within the cell, for the purpose of protecting the body. Traditionally, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most commonly used transaminases in the assessment of hepatic injury. AST is a non-specific liver marker because it would also increase in other diseases such as heart lesion, skeletal muscle disease, renal lesions or red cell lesion, especially in hemolytic disease. ALT possesses a higher specificity than AST, but also a longer half-life. It has been reported that GSTA1 has a short half-life of 1 h, and that content would increase earlier than for ALT for several hours at the early stage of liver injury and decrease earlier during convalescence.[7] Our previous studies have demonstrated that GSTA1 is a more sensitive and more accurate indicator than ALT.[8] The decreased detection of GSTA1 in liver while increased in serum is related to the alcoholism in mice.[9]

In the present study, we evaluated the protective effects of ethanol extracts of FS on hepatotoxicity induced by APAP treatment *in vitro* and *in vivo*, and explored the further usage and effect of FS on the prevention of hepatopathy and the possibility of GSTA1 being used as a maker of hepatic injury.

2. Methods

2.1. Reagents

Commercial kits used for determining ALT, AST, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), nitric oxide (NO) and glutathione peroxidase (GSH-Px) were purchased from the Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). The APAP was obtained from Yongan Chemical Industry (Anhui, China).Type IV collagenase, dimethyl sulfoxide (DMSO), insulin, transferrin, heparin, dexamethasone, and trypan blue were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). The GSTA1 detection kit was purchased from American Rapidbio Company, West Hills, CA, USA. *Folium Syringae* and Silymarin was purchased from the Harbin Jiacheng Dispensary (Harbin, China).

2.2. Primary hepatocytes

Primary hepatocytes were obtained by the inverse perfusion method improved on by Seglen's two-step perfusion *in situ*, according to an earlier study performed by our group.[10] The hepatocytes were selected and seeded in a culture plate containing 24 wells (5×10^4 cell/well) for 6 h, and then the culture media was replaced by growing culture media without serum for 24 h before APAP treatment. All cultures were maintained at 37 °C in a moist atmosphere containing a 5% CO_2.

2.3. Animals

Male Kunming mice (weighing 18–22 g), procured from the Central laboratory of Harbin Pharmaceutical Group Co. (Harbin, China) were used. We ultimately used only male mice because of their constant metabolism compared with the variations in the female physiology. All animals were housed in standard environmental conditions, maintained at a temperature of 20 ± 2 °C a relative humidity of 40–60%, and unlimited access to distilled water and a standard rodent diet. The mice were acclimatized to laboratory condition for a week prior to the experiment. All procedures involving animals that complied with the China National Institutes of Healthy Guidelines for the Care and Use of Laboratory Animals.

2.4. FS ethanol extracts preparation

FS were extracted with ethanol and were subjected to high performance liquid chromatography (HPLC) method for the control quality, using rutin (one of the principal constituents of FS) as a reference. Thereafter, FS leaves were crushed to a fine powder with a universal grinder, then the FS powder (500 g) was extracted with 5000 mL of ethanol (70%, v/v) in ultrasonic mode for 60 min. After extraction, the solvent was filtered and evaporated using a rotavapor at 60 ± 2 °C until the extracts concentrated to a paste, and then were dried by a vacuum drying oven at 35 ± 2 °C, with a resulting flour. The flour was dissolved in saline or culture medium before administration to mice or hepatocytes. The FS ethanol extracts accounted for 30% of the original leaves of FS.

2.5. Protective effects on APAP-induced cytotoxicity

The primary hepatocytes were randomly divided into five groups ($n = 6$): 1) control group; 2) APAP model group; 3) high concentration of FS ethanol extracts group (FS-H, 200 μg/mL); 4) middle concentration of FS ethanol extracts group (FS-M, 150 μg/mL); and 5) low concentration of FS ethanol extracts group (FS-L, 100 μg/mL), respectively. The FS groups were pretreated with different doses of FS ethanol extracts, and the rest of the groups were treated with an equal volume of culture medium. After 12 h, the culture medium was replaced, and each group was treated with 10 mmol/L APAP except for the control group. After 10 h, the supernatant was collected for the determination of ALT, AST, NO and GSTA1; additionally, the hepatocytes were gathered for the determination of MDA, SOD and GSH.

2.6. Protective effects on APAP-induced acute hepatic injury

The mice were randomly divided into six groups, with eight mice per group as follows: control group, APAP model group, high dose FS ethanol extracts group (FS-H, 200 mg/kg), middle dose FS ethanol extracts group (FS-M, 150 mg/kg), low dose FS ethanol extracts group (FS-L, 100 mg/kg) and positive group (Silymarin 200 mg/kg), respectively. The control group and model group were administered saline, while other groups were administered FS ethanol extracts and Silymarin by gavage daily for 7 days. Then, 16 h after the final administration, each group was treated with APAP at a dose of 200 mg/kg of body weight except for the control group and fasted for 12 h. Blood was obtained from the eyeballs and serum, and separated for later measurement. Liver samples were also collected and rinsed with ice cold saline. The liver samples were stored at −80 °C until used for further studies, and a portion of each liver sample was fixed in 10% formalin for histopathological analysis.

2.7. Determination of indices

ALT, AST, MDA, SOD, NO, GSH, GSH-Px, GSTA1 were determined using detection kits according to the manufacturer's instructions.

2.8. Histopathological analysis

The liver tissue from different groups were collected and fixed in 10% formalin, dehydrated in graduated ethanol 50%–100%, cleared in xylene, and embedded in paraffin. Sections 5 μm in thickness were then prepared, stained with hematoxylin and eosin dye, and microscopically examined for histopathological changes.

2.9. Statistical analysis

The data were expressed as mean ± standard deviation (SD). The differences between different groups were analyzed using one-way analysis of variance (ANOVA). $p < 0.01$ was considered as statistically significant, and $p < 0.05$ was considered as statistically marked with Tukey's multiple comparison test. Statistical analyses were carried out using SPSS version 19.0 software (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Protective effects of FS in vitro

3.1.1. Effects of FS on hepatocyte culture supernatant ALT and AST activities

The results of the hepatoprotective effects of FS on hepatocyte culture supernatant ALT and AST activities are presented in Fig. 1. Compared with the model group, ALT activity markedly decreased ($p < 0.05$) when treated with a middle concentration of FS ethanol extracts (150 μg/mL), and both ALT and AST activities significantly decreased ($p < 0.01$) with a high concentration of FS ethanol extracts (200 μg/mL).

3.1.2. Effects of FS on APAP-induced peroxidation in vitro

It was observed that GSH, MDA, NO and SOD significantly changed ($p < 0.01$) in the model group, which indicated that the model was successful. The results of the hepatoprotective effects of FS on hepatocyte culture supernatant NO and hepatocytes MDA, SOD and GSH are presented in Table 1. Compared with the model group, MDA decreased but SOD increased markedly ($p < 0.05$) with a low concentration of FS ethanol extracts (100 μg/mL). Additionally, NO and MDA significantly decreased while SOD and GSH increased ($p < 0.01$) with a middle or high concentration of FS ethanol extracts (150 μg/mL or 200 μg/mL).

3.1.3. GSTA1 content in hepatocyte culture supernatant

Compared with the control group, the GSTA1 level in hepatocyte culture supernatant significantly increased ($p < 0.01$) after the treatment with APAP in the model group. Compared with the model group, the GSTA1 level significantly decreased ($p < 0.01$) with three different concentrations of FS ethanol extracts (100 μg/mL, 150 μg/mL or 200 μg/mL). The results of the hepatoprotective effects of FS on hepatocyte culture supernatant GSTA1 content are presented in Fig. 2.

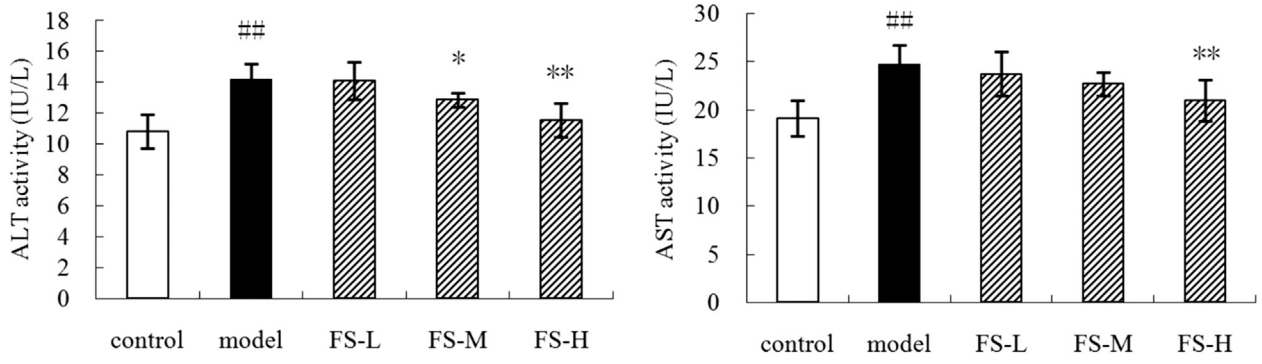

Fig. 1. The changes of ALT and AST activities in hepatocyte culture supernatant. Values expressed as mean ± standard deviation in each group. $^{\#\#}p < 0.01$ compared with the control group;*$p < 0.05$, **$p < 0.01$ compared with the model group; n = 6. ALT = alanine aminotransferase; AST = aspartate aminotransferase; control = control group; FS-H = group administrated with FS ethanol extracts at a concentration of 200 μg/mL; FS-L = group administrated with FS ethanol extracts at a concentration of 100 μg/mL; FS-M = group administrated with FS ethanol extracts at a concentration of 150 μg/mL; model = APAP model group.

Table 1
The changes of GSH, MDA, NO and SOD in primary hepatocytes.

Group	GSH	MDA	NO	SOD
	(μmol/g prot)	(nmol/mg prot)	(μmol/L)	(U/mg prot)
Control	10.31 ± 0.45	1.01 ± 0.06	5.54 ± 0.74	22.34 ± 0.86
Model	8.49 ± 0.58##	1.28 ± 0.05##	9.61 ± 1.23##	18.64 ± 0.51##
FS-L	8.72 ± 0.40	1.18 ± 0.05*	8.63 ± 0.60**	19.56 ± 0.90*
FS-M	9.36 ± 0.61**	1.11 ± 0.10**	6.54 ± 0.79**	20.60 ± 0.83**
FS-H	9.72 ± 0.77**	0.99 ± 0.07**	5.66 ± 0.84**	21.71 ± 0.58**

Values expressed as the mean ± standard deviation in each group; n = 6. ##$p < 0.01$ compared with the control group; *$p < 0.05$, **$p < 0.01$ compared with the model group. Control = control group; FS-H = group administrated with FS ethanol extracts at a dose of 200 μg/mL; FS-L = group administrated with FS ethanol extracts at a dose of 100 μg/mL; FS-M = group administrated with FS ethanol extracts at a dose of 150 μg/mL; GSH = glutathione; MDA = malondialdehyde; model = APAP model group; NO = nitric oxide; SOD = superoxide dismutase.

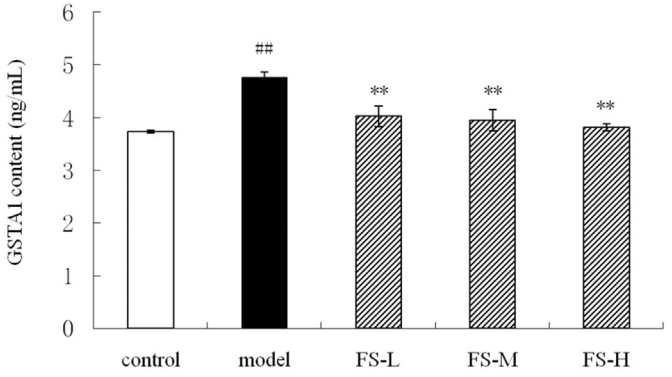

Fig. 2. The changes of GSTA1 content in hepatocyte culture supernatant. Values expressed as mean ± standard deviation in each group; n = 6. ##$p < 0.01$ compared with the control group; **$p < 0.01$ compared with the model group. Control = control group; FS-H = group administrated with FS ethanol extracts at a concentration of 200 μg/mL; FS-L = group administrated with FS ethanol extracts at a concentration of 100 μg/mL; FS-M = group administrated with FS ethanol extracts at a concentration of 150 μg/mL; GSTA1 = glutathione S-transferase alpha 1; model = APAP model group.

3.2. Protective effects of FS in vivo

3.2.1. Effects of FS on serum ALT and AST activities

Compared with the control group, as can be seen in Fig. 3, ALT and AST activities were significantly increased ($p < 0.01$) after administration with APAP, indicating that the model was successful. Compared with the model group, for animals pretreated with low dose of FS ethanol extracts (100 mg/kg) before APAP exposure, AST activity significantly decreased ($p < 0.01$). For those animals treated with middle and high dose of FS ethanol extracts (150 mg/kg and 200 mg/kg), both ALT and AST significantly decreased ($p < 0.01$), especially in high dose FS group, and ALT and AST activities were near those levels of the positive group (Silymarin).

3.2.2. Effects of FS on APAP-induced peroxidation in vivo

The results of the hepatoprotective effects of FS on liver GSH, GSH-Px, MDA, NO and SOD levels are presented in Table 2. All the indices significantly changed with APAP treatment in that NO and MDA increased, and SOD, GSH, GSH-Px decreased. Compared with the model group, GSH-Px, MDA, and NO levels significantly changed ($p < 0.01$) under the low dose of FS ethanol extracts administration (100 mg/kg), and levels of these five indices significantly changed ($p < 0.01$) under the middle and high dose of FS ethanol extracts administration (150 mg/kg and 200 mg/kg). Also, these five indices levels in high dose FS group were similar to those in Silymarin group.

3.2.3. GSTA1 contents in serum and liver

When compared to the control group, a significant descendent ($p < 0.01$) of GSTA1 content in liver (Fig. 4A) and ascendant ($p < 0.01$) of GSTA1 content in serum (Fig. 4B) were observed when APAP was administered. The results of the hepatoprotective effects of FS on serum and liver GSTA1 contents are presented in Fig. 4. Interestingly, these changes were significantly ameliorated by administration of three doses of FS

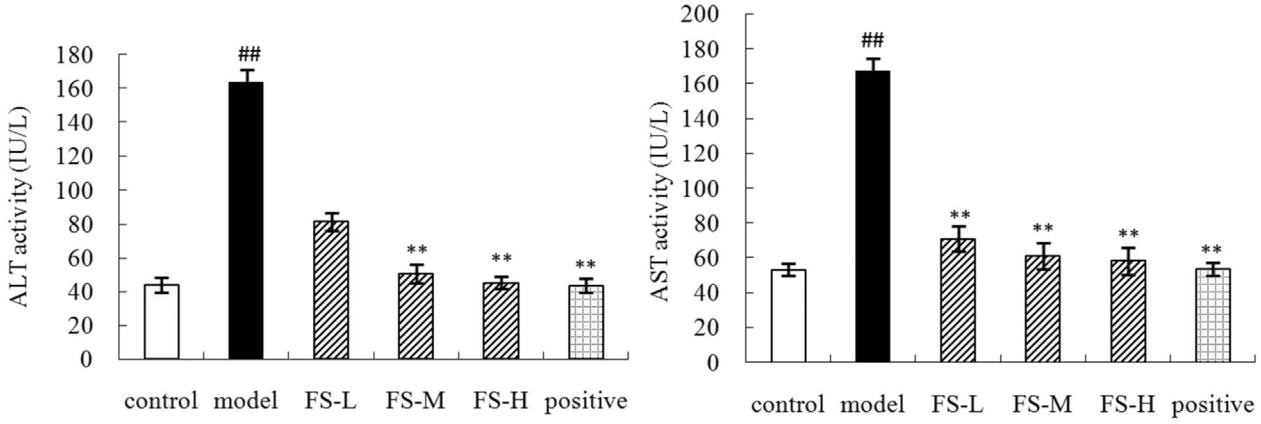

Fig. 3. The changes of ALT and AST activities in the serum of mice with APAP-induced hepatic injury. Values expressed as mean ± standard deviation in each group; n = 8. ##$p < 0.01$ compared with the control group; **$p < 0.01$ compared with the model group. ALT = alanine aminotransferase; AST = aspartate aminotransferase; control = control group; FS-H = group administrated with FS ethanol extracts at a dose of 200 mg/kg; FS-L = group administrated with FS ethanol extracts at a dose of 100 mg/kg; FS-M = group administrated with FS ethanol extracts at a dose of 150 mg/kg; model = APAP model group; positive = group administrated with Silymarin at a dose of 200 mg/kg.

Table 2
The changes of GSH, GSH-Px, MDA, NO, and SOD in mice.

Group	GSH	GSH-Px	MDA	NO	SOD
	(μmol/g prot)	(U/mg prot)	(nmol/mg prot)	(μmol/L)	(U/mg prot)
Control	11.86 ± 0.52	306.07 ± 9.69	8.14 ± 0.52	4.97 ± 0.30	129.42 ± 9.32
Model	7.33 ± 0.63##	214.27 ± 11.48##	11.45 ± 0.73##	7.77 ± 0.44##	80.44 ± 14.39##
FS-L	8.05 ± 1.01	256.04 ± 16.27**	9.21 ± 0.36**	6.08 ± 0.53**	98.47 ± 10.32
FS-M	10.34 ± 1.45**	288.02 ± 12.81**	8.77 ± 0.38**	5.60 ± 0.34**	116.86 ± 12.98**
FS-H	11.31 ± 0.67**	297.37 ± 8.71**	8.36 ± 0.43**	5.12 ± 0.15**	126.84 ± 14.12**
Positive	11.45 ± 0.52**	301.72 ± 8.39**	8.17 ± 0.30**	5.05 ± 0.27**	127.70 ± 8.86**

Values expressed as the mean ± standard deviation in each group; n = 8. ##$p < 0.01$ compared with the control group; **$p < 0.01$ compared with the model group. Control = control group; FS-H = group administrated with FS ethanol extracts at a dose of 200 mg/kg; FS-L = group administrated with FS ethanol extracts at a dose of 100 mg/kg; FS-M = group administrated with FS ethanol extracts at a dose of 150 mg/kg; GSH = glutathione; GSH-Px = glutathione peroxidase; MDA = malondialdehyde; model = APAP model group; NO = nitric oxide; positive = group administrated with Silymarin at a dose of 200 mg/kg; SOD = superoxide dismutase.

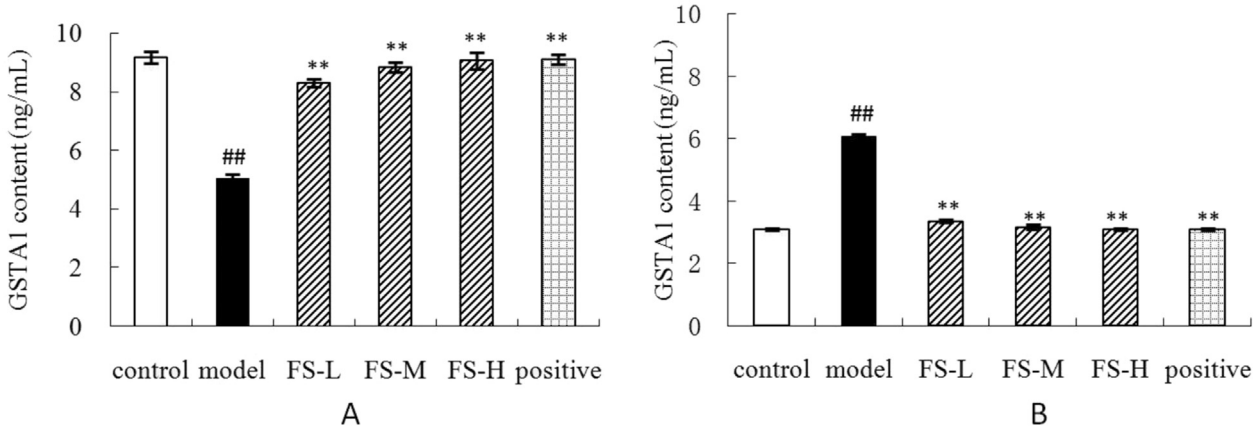

Fig. 4. The changes of GSTA1 contents in liver (A) and serum (B) of mice with APAP-induced hepatic injury. Values expressed as mean ± standard deviation in each group; n = 8. ##$p < 0.01$ compared with the control group; **$p < 0.01$ compared with the model group. Control = control group; FS-H = group administrated with FS ethanol extracts at a dose of 200 mg/kg; FS-L = group administrated with FS ethanol extracts at a dose of 100 mg/kg; FS-M = group administrated with FS ethanol extracts at a dose of 150 mg/kg; GSTA1 = glutathione S-transferase alpha 1; model = APAP model group; positive = group administrated with Silymarin at a dose of 200 mg/kg.

ethanol extracts, and administration of high dose of FS ethanol extracts (200 mg/kg) before APAP exposure; thereafter, the behaviors of GSTA1 were comparable to the Silymarin group.

3.2.4. Histopathological analysis

In the control group, normal hepatic cells were observed with distinct lobuli hepatis. Following APAP treatment, the cells appeared necrocytosis and with hyperchromatic nuclei. The hepatic cells of mice with Silymarin treatment appeared to have normal lobular architecture. After the administration of high dose of FS ethanol extracts (200 mg/kg), some blutene chloride were observed around the central veins, with little necrocytosis or hyperchromatic nuclei. The extent of hepatoprotection afforded by the high dose of FS ethanol extracts against the APAP-induced liver injury was similar to the Silymarin group.

4. Discussion

Folium Syringae has been used to treat inflammatory diseases in China, particularly intestinal inflammations, such as acute enteritis and bacillary dysentery, and upper respiratory tract infections. Recently, studies have shown that *Folium Syringae* could scavenge free radicals[11] and inhibit superoxidation.[12] An overdose of APAP can result in severe liver injury and formation of NAPQI, a toxic metabolic intermediate, mainly via the cytochrome P4502E1 (CYP2E1) enzyme pathway.[13] NAPQI is a very active free radical which can readily oxidize lipids; consequently, it triggers the process of lipid peroxidation to cause the damage in liver cells.[14] The present study reports the potential hepatoprotective effects of *Folium Syringae* against liver injury produced by APAP in primary hepatocytes and mice.

Aminotransferase activities are traditional markers of cellular leakage and loss of functional integrality of hepatocyte membranes. These enzymes normally exist in cytoplasm, but with liver injury they can enter the circulatory system due to toxicity-mediated altered permeability of the cellular membrane.[15] Hence, high levels of ALT, AST in blood and supernatant indicate a severe injury of hepatocytes. In this experiment, APAP exposure caused a significant increasing of ALT and AST both *in vitro* and *in vivo*. However, with administration of FS ethanol extracts (200 μg/mL *in vitro*,

200 mg/kg *in vivo*), this significantly prevented hepatic injury induced by APAP and decreased the release of ALT and AST. These data support that FS could be developed to prevent or ameliorate liver injury induced by APAP.

GSH, as the first line of defense against free radicals, is the chief antioxidant and redox regulator and thus an important preventive agent for many diseases, including liver disease.[16] GSH-Px, the major peroxide detoxification enzyme, may function inefficiently under conditions of glutathione depletion.[17] SOD is considered as antioxidant defense enzyme against the potential free radicals released by the toxic influence that causes oxidative stress, which can inhibit the initiation of lipid peroxidation.[18] When liver injury occurs, these three indices in liver would decrease. In this experiment, with high concentration of FS ethanol extracts, GSH and SOD levels increased by 1.15 times *in vitro* and 1.55 times *in vivo*, suggesting that the hepatoprotective effects of FS could be due to its antioxidant properties. Also, GSH-Px level increased by 1.35 times in mice pre-treated with high dose of FS, which demonstrated that FS alleviated peroxidation caused by APAP.

MDA is one of the lipid peroxidation products, which has been used as a biomarker of lipid peroxidation for several decades.[19] NO is a highly reactive oxidant that is produced through the action of inducible nitric oxide synthase. The production of excessive NO could cause hepatocellular damage by oxidizing and nitrating cellular macromolecules via a reaction with a superoxide anion.[20] The present study showed that levels of MDA and NO significantly decreased with high dose of FS administration, which were reduced nearly to the level of the positive group. We can also clearly find that the same changes occurred in hepatocytes *in vitro*. The results indicated that FS could protect hepatocytes against APAP exposure by enhancing antioxidant capabilities.

GSTA1 has the function for detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress.[21] In addition, GSTA1 can exhibit the activity of glutathione peroxidase by protecting the cells from reactive oxygen species and the products of peroxidation.[22] In this study, APAP-induced unusual expression of GSTA1 (down-regulation in liver and up-regulation in serum) was reversed by FS ethanol extracts (from 9.06 ng/mL to 5.05 ng/mL in liver, 6.05 ng/mL to 3.10 ng/mL in serum) and the hepatoprotective effect of FS was close to Silymarin. This suggested that FS might protect the liver from reactive oxygen species injury through regulation of GSTA1. The changes of GSTA1 in serum and liver indicated that GSTA1 had a vital significance in the evaluation of hepatic injury. Similarly, in primary hepatocytes, GSTA1 content significantly increased after APAP exposure, yet significantly decreased with three doses of FS ethanol extracts. Compared with ALT and AST activities, GSTA1 content indicated a similar trend, and GSTA1 content significantly changed when hepatocytes treated with FS ethanol extracts at the concentration of 100 μg/mL. But ALT and AST activities did not change at this concentration. This suggested that GSTA1was more sensitive than ALT and AST, and could be used as a new marker of liver injury.

In conclusion, our findings clearly demonstrate that pretreatment with FS ethanol extracts could contribute to scavenger free radicals and enhance the antioxidation activities *in vivo* and *in vitro*. This protecting ability of FS was due to, at least partially, its modulation on detoxification enzymes (GSTA1, GSH-Px) and antioxidative enzymes (SOD, GSH) that, simultaneously, repressed the production of free radicals and subsequent liver damage. Therefore, GSTA1 is more sensitive than ALT and AST, and it could be a vital enzyme against APAP-induced liver injury.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant No. 31472241).

References

1. Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M. Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential. *BMC Complement Altern Med* 2014;**14**:225.
2. Rodrigues TG, Fernandes Jr A, Sousa JP, Bastos JK, Sforcin JM. In vitro and in vivo effects of clove on pro-inflammatory cytokines production by macrophages. *Nat Prod Res* 2009;**23**:319–26.
3. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J Pharmacol Exp Ther* 1973;**187**:211–7.
4. Dahlin DC, Miwa GT, Lu AY, Nelson SD. N-acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. *Proc Natl Acad Sci U. S A* 1984;**81**:1327–31.
5. Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. *Mutat Res* 2001;**482**:21–6.
6. Ng L, Nichols K, O'Rourke K, Maslen A, Kirby GM. Repression of human GSTA1 by interleukin-1 beta is mediated by variant hepatic nuclear factor-1C. *Mol Pharmacol* 2007;**71**:201–8.
7. Chouker A, Martignoni A, Schauer RJ, Dugas M, Schachtner T, Kaufmann I, et al. Alpha-gluthathione S-transferase as an early marker of hepatic ischemia/reperfusion injury after liver resection. *World J Surg* 2005;**29**:528–34.
8. Liu FP, Lin YX, Li Z, Ma X, Han Q, Liu YS, et al. Glutathione S-transferase A1 (GSTA1) release, an early indicator of acute hepatic injury in mice. *Food Chem Toxicol* 2014;**71**:225–30.
9. Liu FP, Ma X, Li MM, Li Z, Han Q, Li R, et al. Hepatoprotective effects of Solanum nigrum against ethanol-induced injury in primary hepatocytes and mice with analysis of glutathione S-transferase A1. *J Chin Med Assoc* 2016;**79**:65–71.
10. Chang YC, Liu FP, Ma X, Li MM, Li R, Li CW, et al. Glutathione S-transferase A1-a sensitive marker of alcoholic injury on primary hepatocytes. *Hum Exp Toxicol* 2017;**36**:386–94.
11. Gülçin İ. Fe 3+ −Fe 2+ transformation method: an important antioxidant assay. *Methods Mol Biol* 2015;**208**:233–46.
12. Booyens J, Labuschagne MC, Thantsha MS. In vitro antibacterial mechanism of action of crude garlic (allium sativum) clove extract on selected probiotic bifidobacterium species as revealed by SEM, TEM, and SDS-PAGE analysis. *Probiotics Antimicrob Proteins* 2013;**6**:82–7.
13. Savides MC, Oehme FW. Acetaminophen and its toxicity. *J Appl Toxicol* 1983;**3**:96–111.
14. Moore M, Thor H, Moore G, Nelson S, Moldeus P, Orrenius S. The toxicity of acetaminophen and N-acetyl-p-benzoquinone imine in isolated hepatocytes is associated with thiol depletion and increased cytosolic Ca2+. *J Biol Chem* 1985;**260**:13035–40.
15. Morel F, Schulz WA, Sies H. Gene structure and regulation of expression of human glutathione S-transferases alpha. *Biol Chem Hoppe Seyler* 1994; **375**:641–9.

16. Wu GY, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr* 2004;**134**:489−92.
17. Zakowski JJ, Tappel AL. Purification and properties of rat liver mitochondrial glutathione peroxidase. *Biochim Biophys Acta* 1978;**526**:65−76.
18. Hsu YW, Tsai CF, Chen WK, Lu FJ. Protective effects of seabuckthorn (Hippophae rhamnoides L.) seed oil against carbon tetrachloride-induced hepatotoxicity in mice. *Food Chem Toxicol* 2009;**47**:2281−8.
19. Lykkesfeldt J. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. *Clin Chim Acta* 2007;**380**:50−8.
20. Upur H, Amat N, Blazekovic B, Talip A. Protective effect of Cichorium glandulosum root extract on carbon tetrachloride-induced and galactosamine-induced hepatotoxicity in mice. *Food Chem Toxicol* 2009;**47**:2022−30.
21. Stenberg G, Bjornestedt R, Mannervik B. Heterologous expression of recombinant human glutathione transferase A1-1 from a hepatoma cell line. *Protein Expr Purif* 1992;**3**:80−4.
22. Wills PJ, Asha VV. Protective effect of Lygodium flexuosum (L.) Sw. extract against carbon tetrachloride-induced acute liver injury in rat. *J Ethnopharmacol* 2006;**108**:320−6.