

# The protection of glycyrrhetic acid (GA) towards acetaminophen (APAP)-induced toxicity partially through fatty acids metabolic pathway

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## Abstract

**Background:** Acetaminophen (APAP)-induced liver toxicity remains the key factor limiting the clinical application of APAP, and herbs are the important sources for isolation of compounds preventing APAP-induced toxicity.

**Aims:** To investigate the protection mechanism of glycyrrhetic acid towards APAP-induced liver damage using metabolomics method.

**Methods:** APAP-induced liver toxicity model was made through intraperitoneal injection (i.p.) of APAP (400 mg/kg). Glycyrrhetic acid was dissolved in corn oil, and intraperitoneal injection (i.p.) of glycyrrhetic acid (500 mg/kg body weight) was performed for 20 days before the injection of APAP. UPLC-ESI-QTOF MS was employed to analyze the metabolomic profile of serum samples.

**Results:** The pre-treatment of glycyrrhetic acid significantly protected APAP-induced toxicity, indicated by the histology of liver, the activity of ALT and AST. Metabolomics showed that the level of palmitoylcarnitine and oleoylcarnitine significantly increased in serum of APAP-treated mice, and the pre-treatment with GA can prevent this elevation of these two fatty acid-carnitines.

**Conclusion:** Reversing the metabolism pathway of fatty acid is an important mechanism for the protection of glycyrrhetic acid towards acetaminophen-induced liver toxicity.

**Keywords:** Glycyrrhetic acid (GA), acetaminophen (APAP), metabolomics, fatty acid

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## Introduction

The human respiratory system is divided into the upper

and lower respiratory tract. The upper respiratory tract comprises the nasal cavity, pharynx, and larynx, and the lower respiratory tract is consisted of the trachea, bronchi, and the lungs. The respiratory system diseases contain upper respiratory tract infections, lower respiratory tract infections, asthma, pneumothorax, chronic obstructive pulmonary disease, pulmonary fibrosis, tuberculosis, and even cancers<sup>1,2</sup>. Searching effective drugs for respiratory system diseases is very important. Glycyrrhetic acid, also called as enoxolone, is a pentacyclic triterpenoid derivative of the beta-amyrin type obtained from the hydrolysis of glycyrrhizic acid which is an important herbal ingredient isolated from herb licorice<sup>3</sup>. Glycyrrhetic acid has exerted therapeutic role towards respiratory diseases. For example, glycyrrhetic acid has been demonstrated to show therapeutic role towards non-small cell lung cancer<sup>4</sup>.

The adverse effects of acetaminophen (APAP) remain the major reason in limiting the clinical application of

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APAP which is one of the most important over-the-counter analgesic and antipyretic drugs. The reason for APAP-induced adverse effects is the bioactivation of APAP into the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) which is the key substance basis for APAP-induced toxicity. Metabolomics-based system biology method has been widely used to elucidate the detailed mechanism of APAP-induced liver toxicity. The experiment performed by Chen et al. used metabolomics method to compare the metabolism behavior of APAP between wild type and CYP2E1-null mice, and found several new metabolites of APAP among which APAP dimer has high correlation with the toxicity of APAP<sup>5</sup>. Serum metabolomics analysis showed that APAP induced mitochondria damage through disrupting oxidative process of fatty acids<sup>6</sup>. Furthermore, the protective role of PPAR alpha agonist towards APAP-induced toxicity supported the importance of fatty acid metabolism in APAP-induced toxicity<sup>7</sup>.

Given that the disruption of fatty acids metabolism is an important reason for APAP-induced toxicity, the present study aimed to investigate the influence of glycyrrhetic acid towards acetaminophen (APAP)-induced fatty acids metabolism.

## **Materials and methods**

### **Chemicals and reagents**

Acetaminophen (APAP), acetonitrile, and formic acid were purchased from Sigma-Aldrich (St. Louis, Mo., USA).

### **Animal experiment and sample preparation**

The animal experiment was performed under the agreement approved by the committee of Yantai Yuhuangding Hospital. 12 Male Sv/129 mice were purchased from Beijing Laboratory Animal Research Center (Beijing, China). The mice were maintained under a standard 12-h light/12-h dark cycle with food and water ad libitum. APAP-induced liver toxicity model was made through intraperitoneal injection (i.p.) of APAP (400 mg/kg). Glycyrrhetic acid was dissolved in corn oil, and intraperitoneal injection (i.p.) of glycyrrhetic acid (500 mg/kg body weight) was performed for 20 days before the injection of APAP. Blood was collected at 6 hour after the administration of APAP using BD Microtainer™ serum separator tubes (Franklin Lakes, NJ)

by retro-orbital bleeding. After the sacrifice of mice, the livers were excised and used for histology analysis.

### **Determination of activity of aspartate aminotransferase (AST) and alanine transaminase (ALT)**

The mechanism for determination of AST and ALT activity is to the determination of reaction velocity for the catalysis process of oxidation of NADH to NAD<sup>+</sup>. During this process, 200  $\mu$ L of ALT or AST assay buffer was added to catalyze the reaction of 1  $\mu$ L of serum. The whole catalytic process was monitored using the wavelength at 340 nm.

### **Serum metabolomics analysis**

10  $\mu$ L of serum was firstly mixed with 190  $\mu$ L of 80% acetonitrile, and the centrifugation at 14,500 rpm was used to deproteinization. 5  $\mu$ L of the supernatant was added into the vials to undergo UPLC-QTOF-MS analysis. Acquity UPLC BEH C18 column (1.7  $\mu$ m, 2.1 $\times$ 50 mm, Waters Corp.) was used to separate the compounds. The elution phase was consisted of water with 0.2% formic acid (A) and acetonitrile with 0.5% formic acid (B). The gradient elution condition was used as followed: 0-3 min, 98%-70% A; 3-8 min, 70%-5% A; 8-9 min, 5% A. Finally, the elution condition was brought back to 98% A. The column temperature was maintained at 40°C. Positive mode was used to monitor all the compounds, and the capillary and cone voltages were set as 4 kV and 30 V. 120 and 360°C were selected as source and desolvation temperature, respectively. The elution rate of nitrogen gas was set as 50 L/h and 600 L/h for cone gas and desolvation gas, respectively. For MS/MS fragmentation, the collision energy ranged from 10 to 40 eV.

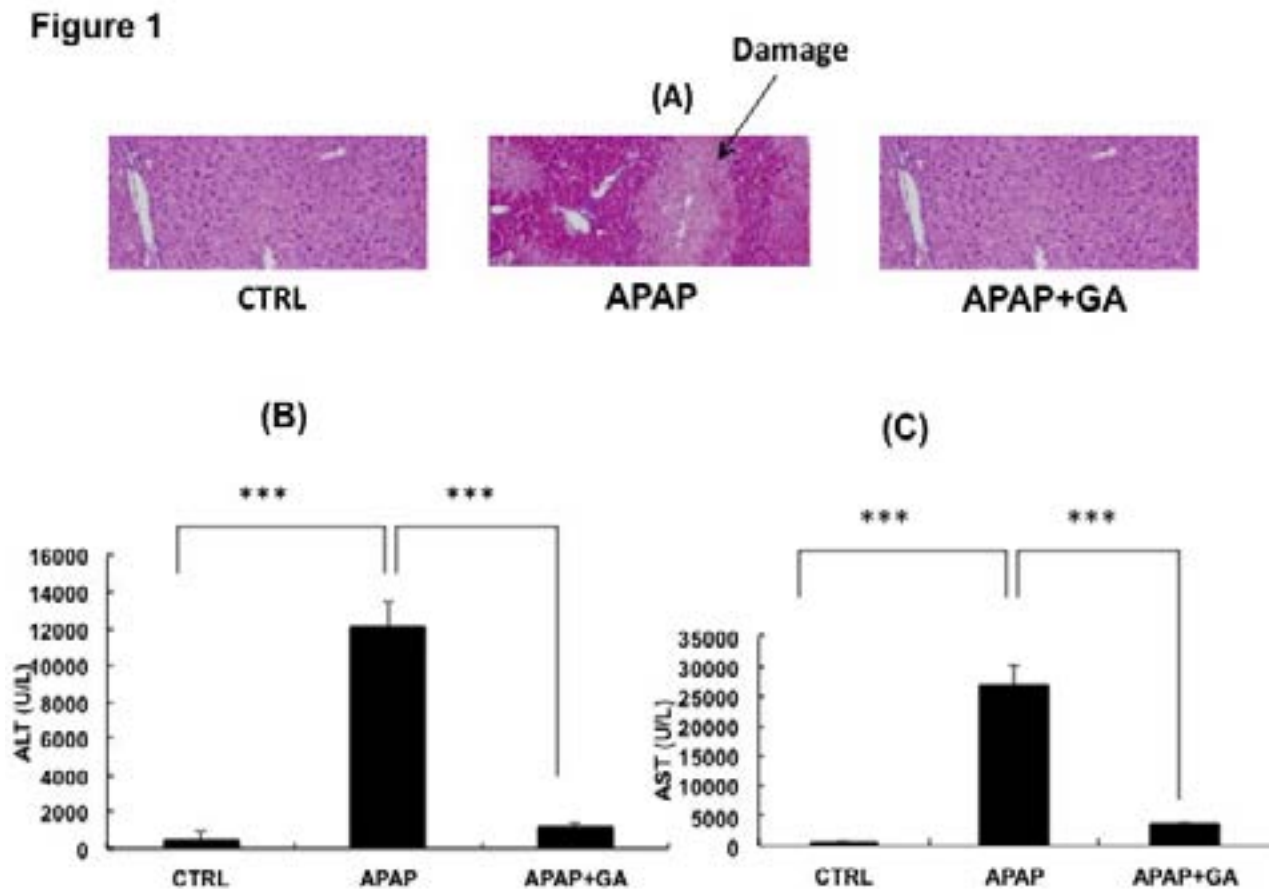
### **Statistical method:**

**The statistical analysis was performed using two-tailed one student's t test.**

### **Results**

The Figure 1 gave the phenotype results. As shown in Figure 1A, compared with the histology of control mice, the livers from APAP-treated mice had some damaged areas, as indicated using arrow sign. The pretreatment with GA can protect the damage induced by APAP treatment (Figure 1A).

**Figure 1** Pre-treatment with glycyrrhetic acid (GA) significantly protected acetaminophen (APAP)-induced liver toxicity. (A) H&E staining to analyze the liver histology from control group, APAP-treated group and GA+APAP-treated group. (B) Comparison of ALT activity in control group, APAP-treated group and GA+APAP-treated group. \*\*\*,  $P < 0.001$ . (C) Comparison of AST activity in control group, APAP-treated group and GA+APAP-treated group. \*\*\*,  $P < 0.001$ .



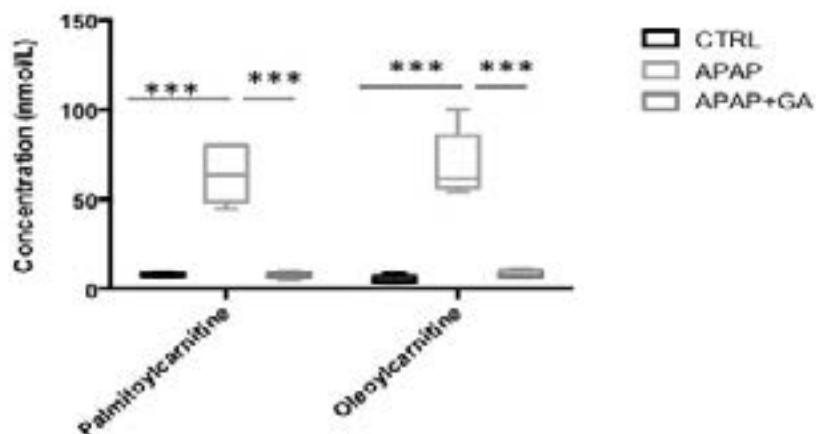
The pre-treatment with GA also prevented the elevation of ALT and AST activity induced by APAP treatment (Figure 1B & C).

The treatment with APAP significantly increased the level of two ions in serum, and these two ions were

identified to be palmitoylecarnitine and oleoylecarnitine (Supplemental Figure 1). The treatment with APAP significantly induced the elevated level of these two compounds ( $p < 0.001$ , Figure 2), and pre-treatment with GA can significantly decrease the level of these two compounds in serum ( $p < 0.001$ , Figure 2).

**Figure 2 Comparison of level of palmitoylcarnitine and oleoylcarnitine in control group, APAP-treated group and GA+APAP-treated group. \*\*\*, P<0.001.**

**Figure 2**



## Discussion

Herbs have become more and more popular in the world, and herbs have exerted therapeutic role towards more and more diseases. The therapeutic behavior of herbs towards APAP-induced liver toxicity has been frequently reported. For example, herb Schisandra Sphenanthera extract has been demonstrated to exhibit therapeutic role towards APAP-induced liver toxicity<sup>8</sup>, and the compound isolated from this herb Schisandrol B has also exerted its protection role towards APAP-induced liver toxicity<sup>9</sup>. Herbal ingredient saikosaponin d (SSd), isolated from traditional Chinese herb Bupleurum falcatum, protects against APAP-induced hepatotoxicity by inhibiting NF-kB and STAT3 signaling<sup>10</sup>.

The present study demonstrated the therapeutic role of glycyrrhetic acid towards APAP-induced liver toxicity through reversing fatty acids metabolic pathway. Given that fatty acids metabolism is a key source for energy production, glycyrrhetic acid-induced recovery of energy production might be the key mechanism for the protection of glycyrrhetic acid towards APAP's toxicity. Targeted metabolomics analysis demonstrated that glycyrrhizin protected APAP-induced liver toxicity through reversing fatty acid metabolism<sup>11</sup>. Therefore,

the same protection mechanism of the compounds with such similar structures might exist. It should be noted that glycyrrhetic acid (GA) can inhibit the activity of CYP2E1 which might also contribute to the protection of GA towards APAP-induced toxicity<sup>12</sup>.

## Conclusion

Reversing the metabolism pathway of fatty acid is an important mechanism for the protection of glycyrrhetic acid towards acetaminophen-induced liver toxicity.

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