Chlorogenic acid prevents acetaminophen-induced liver injury: the involvement of CYP450 metabolic enzymes and some antioxidant signals[*]

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Abstract: Chlorogenic acid (CGA), a polyphenolic compound, is abundant in fruits, dietary vegetables, and some medicinal herbs. This study investigated the prevention of CGA against acetaminophen (AP)-induced hepatotoxicity and its engaged mechanisms. CGA reversed the decreased cell viability induced by AP in L-02 cells *in vitro*. In addition, CGA reduced the AP-induced increased serum levels of alanine/aspartate aminotransferase (ALT/AST) *in vivo*. The effect of CGA on cytochrome P450 (CYP) enzymatic (CYP2E1, CYP1A2, and CYP3A4) activities showed that CGA caused very little inhibition on CYP2E1 and CYP1A2 enzymatic activities, but not CYP3A4. The measurement of liver malondialdehyde (MDA), reactive oxygen species (ROS), and glutathione (GSH) levels showed that CGA prevented AP-induced liver oxidative stress injury. Further, CGA increased the AP-induced decreased mRNA expression of peroxiredoxin (Prx) 1, 2, 3, 5, 6, epoxide hydrolase (Ephx) 2, and polymerase (RNA) II (DNA directed) polypeptide K (Polr2k), and nuclear factor erythroid-2-related factor 2 (Nrf2). In summary, CGA ameliorates the AP-induced liver injury probably by slightly inhibiting CYP2E1 and CYP1A2 enzymatic properties. In addition, cellular important anti-oxidant signals such as Prx1, 2, 3, 5, 6, Ephx2, Polr2k, and Nrf2 also contributed to the protection of CGA against AP-induced oxidative stress injury.

Key words: Chlorogenic acid, Acetaminophen, CYP450, Oxidative stress injury
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1 Introduction

Acetaminophen (AP), a common over-the-counter (OTC) drug, is widely used in clinical practice as an analgesic-antipyretic drug. However, an overdose of AP will cause severe liver injury (Lee, 2004). This adverse effect has become the major cause of drug-induced liver injury in the United States and the United Kingdom (Lee, 2003). *N*-acetylcysteine (NAC) is generally used for the treatment of AP-induced hepatotoxicity, although it has a drawback as it must be given within 8 h after AP intoxication, and it can also cause other side-effects including vomiting, nausea, and even shock (Bajt *et al.*, 2004; Bebarta *et al.*, 2010). Therefore, the need for alternative, more effective, and widely applicable antidotes for AP-induced liver injury is warranted.

Chlorogenic acid (CGA) is the major active ingredient found in many traditional Chinese medicines such as *Folium mori* (Hunyadi *et al.*, 2012) and *Flos Lonicerae japonicae* (Oku *et al.*, 2011), and it is also abundant in some fruits, dietary vegetables (Kasai *et al.*, 2000; Gavrilova *et al.*, 2011), and daily beverages like coffee (del Rio *et al.*, 2010). CGA has been reported to possess anti-bacterial, antioxidant, and anti-carcinogenic properties (Kono *et al.*, 1997; dos Santos *et al.*, 2006). CGA has also been reported to be a potent polyphenolic antioxidant because it contains a certain amount of the R-OH group (Fig. 1a), which can bind with hydroxyl radicals and superoxide anion radicals to protect cells from oxidative injury (Özyürek *et al.*, 2008).

Our previous study has found that CGA can prevent AP-induced liver injury through regulating liver glutathione (GSH) and thioredoxin (Trx) antioxidant systems (Ji *et al.*, 2013). However, whether there is some other antioxidant signals involved in the protection of CGA against AP-induced liver injury has not been identified. Liver CYP2E1, CYP1A2, and CYP3A4 have been reported to be the key metabolic enzymes responsible for metabolizing AP (Laine *et al.*, 2009). In this present study, we observed the protection of CGA against AP-induced hepatotoxicity *in vitro* and *in vivo*, and then observed the potential effects of CGA on CYP2E1, CYP1A2, and CYP3A4 enzymatic properties and their expression, and finally observed the involvement of some antioxidant signals in the protection of CGA against AP-induced liver injury.

2 Materials and methods

2.1 Materials

CGA (5-caffeoylquinic acid, CAS-327-97-9) (Fig. 1a), with 98% purity, was purchased from the Shanghai Hitsanns Co., Ltd. (Shanghai, China). AP, CYP450 inhibitors including sodium diethyldithiocarbamate trihydrate (DDTC), furafylline, and ketoconazole, and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Alanine/aspartate aminotransferase (ALT/AST), malondialdehyde (MDA) analysis kits were pur-

chased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). PrimeScript® RT Master Mix and SYBR® Premix Ex Taq™ were purchased from TaKaRa (Shiga, Japan). Vivid® CYP2E1, Vivid® CYP1A2, and Vivid® CYP3A4 kits, 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA), and Trizol reagent were all purchased from Life Technology (Carlsbad, CA, USA). All other reagents were purchased from Sigma (St. Louis, MO, USA), unless otherwise indicated.

2.2 Cell culture

L-02 cells (Cell Bank, Type Culture Collection of Chinese Academy of Sciences, Shanghai, China), a human normal liver cell line, were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 mg/ml streptomycin in an atmosphere of 5% CO_2 at 37 °C.

2.3 Cell viability assay

Cells were pre-incubated with various concentrations of CGA for 15 min after adherent, and then incubated with AP for another 48 h. After treatments, 500 μg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added, and 4 h later, 10% SDS-5% iso-butanol-0.01 mol/L HCl was added. At last the optical density was measured at 570 and 630 nm, and cell viability (% of control) was calculated.

2.4 CYP450 metabolic enzyme activity assay

The effects of CGA on CYP2E1, CYP1A2, and CYP3A4 enzymatic activities were analyzed by using Vivid® CYP2E1, Vivid® CYP1A2, and Vivid® CYP3A4 kits according to their descriptions. The CYP450 positive inhibitor used for CYP2E1 is DDTC (1 mmol/L), for CYP1A2 it is furafylline (10 μmol/L), for CYP3A4 it is ketoconazole (10 μmol/L), respectively. The reaction rates are obtained by calculating the change in fluorescence per minute. The analysis of the results was performed with a Kinetic Assay Mode using the equation: inhibition of positive inhibitor (%)=[1−(*X*−*B*)/(*A*−*B*)]×100%, where *X*, *A*, and *B* represent the rates of the test compound CGA, solvent control, and positive inhibitor control, respectively.

2.5 Animals and treatments

Male imprinting control region (ICR) mice (specific pathogen-free, SPF), weighing 16–20 g, were

purchased from the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai, China). The mice were fed on a standard laboratory diet and supplied with water *ad libitum* at a temperature of (22±1) °C with a 12-h light and dark cycle and relative humidity of (65±5)%.

The mice were randomly divided into five groups: vehicle control, AP (300 mg/kg), AP+CGA (10 mg/kg), AP+CGA (40 mg/kg), and CGA (40 mg/kg) (with n=8 each group). Mice were pre-administered orally with the vehicle control or 10 or 40 mg/kg CGA for 7 d. On the last day, the mice were given a single dose of AP (300 mg/kg, intragastric (i.g.)) 1 h after administration of CGA. The mice were then killed 4 h after AP injection, and plasma and liver tissue were collected.

2.6 ALT/AST assay

Fresh blood obtained from the mice was allowed to clot for 2 h, and then serum was collected by centrifuging. Serum ALT and AST levels were determined by kits according to the manufacturer's instructions.

2.7 Liver MDA amount assay

Liver homogenates were prepared in cold phosphate buffer solution (PBS). Liver MDA amount was determined by kits according to the manufacturer's instructions, then calculated based on the tissue protein concentration.

2.8 Determination of liver GSH levels

Liver homogenates were prepared in cold PBS. Liver GSH levels were determined by the DTNB assay according to our previous reported method (Liang *et al.*, 2011).

2.9 Determination of liver ROS levels

Liver ROS were measured with the probe H2DCFDA. Briefly, the livers were homogenized in cold PBS, centrifuged at 4 °C, 600g for 10 min. The supernatant was further centrifuged at 4 °C, 10 000g for 15 min, and the precipitation was incubated with 10 μmol/L H2DCFDA in the dark at room temperature for 1 h, and then fluorescence was immediately read at excitation (485±20) nm, emission (525±20) nm by using a spectrophotometer (BioTek Synergy H4). Protein concentrations in the supernatant were assayed, and all the results were calculated as units of fluorescence per milligram of protein.

2.10 Real-time polymerase chain reaction (PCR) analysis

Liver homogenates were prepared in a Trizol regent, and total RNA was extracted according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized by using a PrimeScript™ RT Master Mix kit. Real-time PCR was performed by SYBR Green Premix kits on Step-One-Plus (Applied Biosystems, USA). Relative expression of target genes was normalized to *Actb*, analyzed by the $2^{-\Delta\Delta C_T}$ method and given as a ratio compared to the vehicle control. The primer sequences are listed as Table 1.

Table 1 Primers for real-time PCR

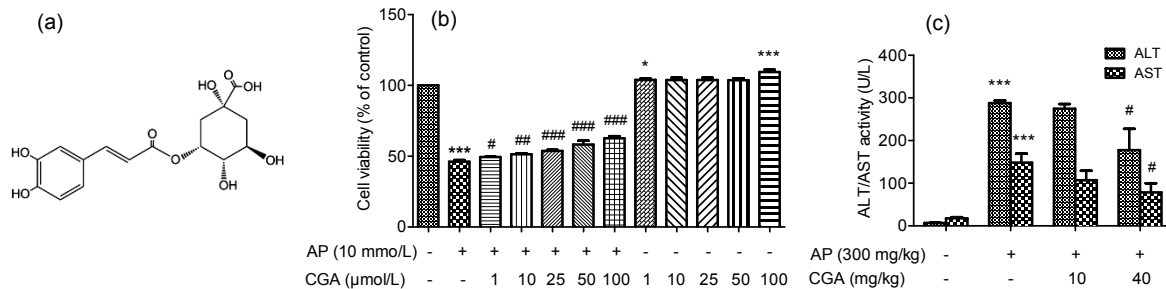| Target	Primer sequence
Actb	F: 5'-TTCGTTGCCGGTCCACACCC-3'
	R: 5'-GCTTTGCACATGCCGGAGCC-3'
Prdx1	F: 5'-CACCCAAGAAACAAGGAGGA-3'
	R: 5'-AAAAAGGCCCCTGAAAGAGA-3'
Prdx2	F: 5'-TGATGAGGGCATTGCTTACAGG-3'
	R: 5'-GAGCGTCCCACAGGTAGGTCAT-3'
Prdx3	F: 5'-TTCCCACTTCAGTCATCTTGCC-3'
	R: 5'-ATGCCAGCACTTTCCAACAG-3'
Prdx4	F: 5'-TTCCTGTTGCGGACCGAAT-3'
	R: 5'-TACACTTGTCCACCAGCGTAGAA-3'
Prdx5	F: 5'-GGAAGGCGACAGACTTATTATTGG-3'
	R: 5'-CCTTCACTATGCCGTTGTCTATCAC-3'
Prdx6	F: 5'-GCACCCACAGAACTTGGCAGAG-3'
	R: 5'-CCAGGCAAGATGATCCTCAACA-3'
Ephx2	F: 5'-CCTTCCAGCTTCGTGTCTGT-3'
	R: 5'-TCCCTCTGGGAATTCCGTCT-3'
Polr2k	F: 5'-TTGTGGAGAGTGTCACACCG-3'
	R: 5'-ACTCCCACATTTCATCGAGCA-3'
Fmo5	F: 5'-CAAGGACGCTGGGCCACTCAA-3'
	R: 5'-CCTGGATGGTATGACGCTGGCT-3'
Mt1	F: 5'-CTCCGTAGCTCCAGCTTCAC-3'
	R: 5'-AGGAGCAGCAGCTCTTCTTG-3'
Mt2	F: 5'-CCGATCTCTCGTCGATCTTC-3'
	R: 5'-AGGAGCAGCAGCCTTTTCTTG-3'
Sod1	F: 5'-CCAGCATGGGTTCCACGTCCAT-3'
	R: 5'-TCCGCCGGGCCACCATGTTT-3'
Sod2	F: 5'-CCACGCGGGCCTACGTGAACA-3'
	R: 5'-TGCAGGCTGAAGAGCGACCTG-3'
Cyp2e1	F: 5'-GGCGCATCGTGGTCCTGCAT-3'
	R: 5'-GGCTGGCCTTTGGTCTTTTTGAGC-3'
Cyp1a2	F: 5'-AGGGACACCTCACTGAATGGC-3'
	R: 5'-GGGTCTTTCCACTGCTTCTCATC-3'
Cyp3a11	F: 5'-GAACTTCTCCTTCCAGCCTTGTAAG-3'
	R: 5'-CGTGGCACAACCTTTAGAACAAT-3'
Nfe2l2	F: 5'-TCTCCTCGCTGGAAAAAGAA-3'
	R: 5'-AATGTGCTGGCTGTGCTTTA-3'

F: forward; R: reverse

2.11 Statistical analysis

Data were expressed as mean±standard error of mean (SEM). The significance of differences between groups was evaluated by one-way analysis of variance (ANOVA) with the least significant difference (LSD) post-hoc test; and $P<0.05$ was considered as statistically significant.

3 Results

3.1 Effect of CGA on AP-induced hepatotoxicity *in vitro* and *in vivo*

As shown in Fig. 1b, AP (10 mmol/L) decreased cell viability dramatically as compared with the control

($P<0.001$), whereas various concentrations of CGA (1, 10, 25, 50, and 100 μmol/L) can reverse the AP-induced decreased cell viability in a concentration-dependent manner ($P<0.05$, $P<0.01$, or $P<0.001$). Further results of serum ALT/AST analysis showed that AP increased serum ALT and AST activities ($P<0.001$), whereas 40 mg/kg CGA reduced the AP-induced increased ALT and AST activities ($P<0.05$ or $P<0.01$) (Fig. 1c).

3.2 Effect of CGA on CYP450 metabolic enzymes

First, we observed whether CGA (40 mg/kg) will affect the liver mRNA expression of CYP2E1, CYP1A2, and CYP3A11 (CYP3A4 in humans) in mice. The results (Fig. 2a) showed that there were no

Fig. 1 Effect of CGA on AP-induced hepatotoxicity *in vitro* and *in vivo*
(a) Chemical structure of CGA. (b) L-02 cells were pre-incubated with CGA for 15 min, and followed by incubation with AP (10 mmol/L) for 48 h. Cell viability was determined by the MTT method. Data are expressed as mean±SEM ($n=5$). (c) Mice were pre-administrated with CGA (10 and 40 mg/kg) for 7 consecutive days, and a single dose of AP (300 mg/kg, i.g.) was given on the final day. Mice were sacrificed 4 h after AP treatment. Data are expressed as mean±SEM ($n=6–8$). The results show that CGA ameliorates AP-induced hepatotoxicity *in vitro* and *in vivo*. [*] $P<0.05$, [***] $P<0.001$ compared with control; [#] $P<0.05$, [##] $P<0.01$, [###] $P<0.001$ compared with only AP treatment
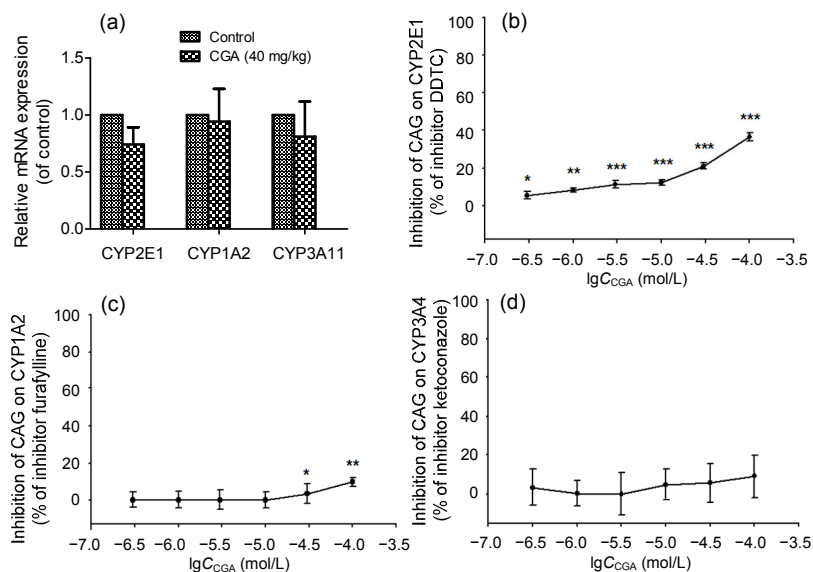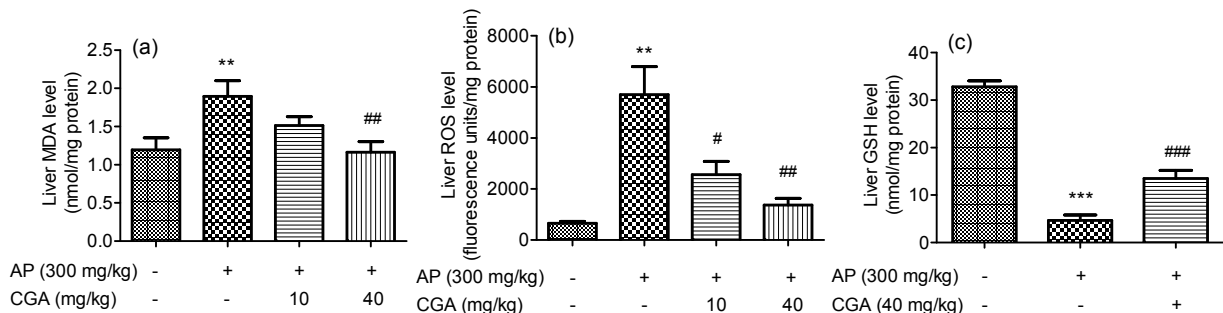

Fig. 2 Effects of CGA on liver mRNA expression and enzymatic activities of CYP2E1, CYP1A2, and CYP3A4
(a) CGA (40 mg/kg) performed no inhibition on liver mRNA expression of CYP2E1, CYP1A2, or CYP3A11. Data are expressed as mean±SEM ($n=6$). (b–d) Inhibition of CGA on the enzymatic activities of CYP2E1 (b), CYP1A2 (c), and CYP3A4 (d). Data are expressed as mean±SEM ($n=6$). [*] $P<0.05$, [**] $P<0.01$, [***] $P<0.001$ compared with solvent control

Fig. 3 Effect of CGA on AP-induced liver oxidative injury
(a) Liver MDA amount; (b) Liver ROS level; (c) Liver GSH amount. Data are expressed as mean±SEM (n=7–8). The results show that CGA ameliorates AP-induced liver oxidative injury. [**] $P<0.05$, [***] $P<0.001$ compared with control; [#] $P<0.05$, [##] $P<0.01$, [###] $P<0.001$ compared with only AP treatment

obvious differences in the expression of CYP2E1, CYP1A2, or CYP3A11 in the liver between the control and CGA-treated mice.

Next, the enzymatic activities of CYP2E1, CYP1A2, and CYP3A4 were performed *in vitro*. As shown in Fig. 2b, CGA displayed less than 40% inhibition of the positive inhibitor control on CYP2E1 enzymatic activity ($P<0.05$, $P<0.01$, or $P<0.001$), and such inhibition was in a concentration-dependent manner. CGA (30 or 100 μmol/L) also had less than a 20% inhibition of positive inhibitor control on CYP1A2 enzymatic activity ($P<0.05$ or $P<0.01$; Fig. 2c). However, CGA had no inhibition on CYP3A4 enzymatic activity (Fig. 2d). These data suggest that the protection of CGA against AP-induced liver injury is probably caused by slightly inhibiting its metabolism.

3.3 Effects of CGA on AP-induced liver oxidative stress injury

As shown in Fig. 3, CGA (40 mg/kg) obviously reduced the increased liver MDA amounts induced by AP (300 mg/kg) ($P<0.01$). Furthermore, the formation of liver ROS was increased in AP (300 mg/kg)-treated mice ($P<0.01$), whereas CGA (10 and 40 mg/kg) reduced such increase ($P<0.01$, $P<0.05$). In addition, the liver GSH amount was decreased in AP (300 mg/kg)-treated mice ($P<0.01$), whereas CGA (40 mg/kg) reversed such a decrease ($P<0.001$).

3.4 Effect of CGA on some oxidative stress genes

As shown in Fig. 4, AP (300 mg/kg) decreased liver mRNA expression of peroxiredoxin (Prx) 1, 2, 3, 5, and 6 ($P<0.05$ or $P<0.01$), but had no effect on Prx4 mRNA expression. CGA (40 mg/kg) reversed the decreased expression of those above genes induced by AP ($P<0.05$ or $P<0.01$).
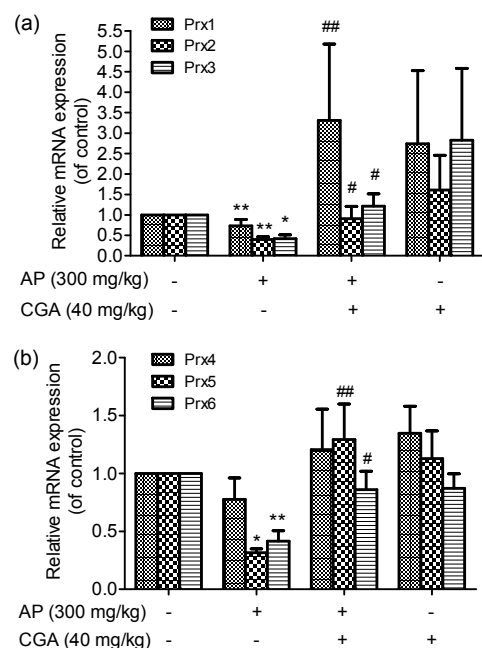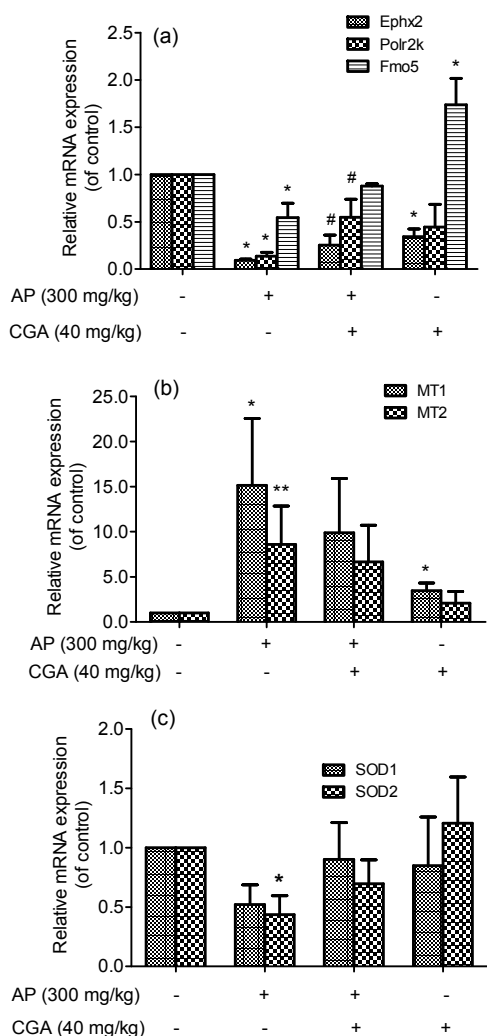

Fig. 4 Effect of CGA on liver Prx1–6 mRNA expression
Liver mRNA expressions of Prx1–3 (a) and Prx4–6 (b) were detected by real-time PCR. Data are expressed as mean±SEM (n=8). [*] $P<0.05$, [**] $P<0.01$ compared with control; [#] $P<0.05$, [##] $P<0.01$ compared with only AP treatment

As shown in Fig. 5a, AP (300 mg/kg) decreased liver mRNA expression of epoxide hydrolase (Ephx) 2, polymerase (RNA) II (DNA directed) polypeptide K (Polr2k), and flavin-containing monooxygenase (Fmo5) ($P<0.05$), whereas CGA (40 mg/kg) reversed the decreased expression of Ephx2 ($P<0.05$) and Polr2k ($P<0.05$), but not Fmo5 ($P>0.05$). However, CGA itself can increase the *Fmo5* gene expression ($P<0.05$).

As shown in Fig. 5b, AP (300 mg/kg) increased liver mRNA expression of metallothionein (MT) 1 ($P<0.05$) and MT2 ($P<0.01$), and CGA itself also slightly increased the MT1 expression. However,

Fig. 5 Effect of CGA on liver mRNA expression of some other oxidative stress genes
Liver mRNA expressions of Ephx2, Polr2k, and Fmo5 (a), MT1 and MT2 (b), SOD1 and SOD2 (c) were detected by real-time PCR. Data are expressed as mean±SEM (*n*=6–8). * *P*<0.05, ** *P*<0.01 compared with control; # *P*<0.05 compared with only AP treatment
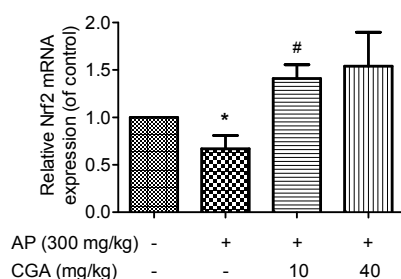
Fig. 6 Effect of CGA on liver Nrf2 mRNA expression
Liver mRNA expression of Nrf2 was detected by real-time PCR. Data are expressed as mean±SEM (*n*=8). * *P*<0.05 compared with control; # *P*<0.05 compared with only AP treatment

CGA had no effect on the AP-induced increased mRNA expression of MT1 and MT2 (*P*>0.05).

As shown in Fig. 5c, AP (300 mg/kg) decreased liver mRNA expression of superoxide dismutase (SOD) 2 (*P*<0.05), but not SOD1 (*P*>0.05). After CGA (40 mg/kg) treatment, the AP-induced decreased expression of SOD2 was not altered (*P*>0.05).

3.5 Effect of CGA on nuclear factor erythroid-2-related factor 2 (Nrf2) mRNA expression

Furthermore, the mRNA expression of Nrf2 was observed. As shown in Fig. 6, AP (300 mg/kg) decreased the liver expression of Nrf2 mRNA (*P*<0.05), but CGA (40 mg/kg) pre-treatment reversed the decreased Nrf2 mRNA expression (*P*<0.05).

4 Discussion

Liver CYP450 enzymes play a pivotal role in the metabolism of xenobiotics. It has been reported that CYP2E1, CYP3A4, and CYP1A2 are the key CYP450 enzymes for the metabolic activation of AP in human liver, and that the metabolic product of AP in the liver will cause serious hepatotoxicity (Raucy *et al.*, 1989; Laine *et al.*, 2009). In addition, AP has been reported to be metabolized into AM404, which acts on transient receptor potential vanilloid 1 (TRPV1), cyclooxygenase (COX), and endogenous cannabinoid system, contributing to antipyretic and analgesic effects of AP (Högestätt *et al.*, 2005).

Our experiment *in vivo* demonstrated that CGA itself had no effect on the mRNA expression of CYP2E1, CYP1A2, and CYP3A11 (CYP3A4 in humans) in the liver. In addition, the results of enzymatic activities *in vitro* showed that CGA displayed weak inhibition on the enzymatic activity of CYP2E1 (less than 40% inhibition of the positive inhibitor), and CYP1A2 (less than 20% inhibition of the positive inhibitor). These results indicate that CGA partly prevented AP-induced liver injury via inhibiting the enzymatic activities of CYP2E1 and CYP1A2. It also indicates that there must be other signals involved in the protection of CGA against AP-induced liver injury.

Our previous study found that CGA prevented AP-induced apoptosis and oxidative stress damage, and liver GSH and Trx antioxidant systems played important roles in such protection (Ji *et al.*, 2013).

The prevention of CGA against AP-induced liver oxidative stress injury was evidenced by the results of MDA, ROS, and GSH levels in this study. Furthermore, we found that some other antioxidant signals also contributed to the protection of CGA against AP-induced liver injury in this study. Prx, a diverse and ubiquitous family of antioxidant proteins, includes six mammalian isoforms: Prx1–6 (Rhee et al., 2005). There are already reports demonstrating that Prx3-knockout increased pyrazole-induced liver oxidative damage (Bae et al., 2012), and Prx6-knockout increased ischemia-reperfusion-induced liver injury (Eismann et al., 2009). In the present study, we found that CGA reversed the decreased mRNA expression of Prx1, 2, 3, 5, and 6 induced by AP. The results suggest that Prx1, 2, 3, 5, and 6 may play important roles in the protection of CGA against AP-induced liver injury.

Ephx2 is a gene responsible for encoding soluble epoxide hydrolase (sEH), which can metabolize endogenous aliphatic and aromatic epoxides, and plays an important role in regulating cellular redox homeostasis (Fretland and Omiecinski, 2000). Polr2k, an important gene in the RNA processing machinery, has been reported to be associated with cancer (Heidenblad et al., 2008), and the role of Polr2k in liver injury is still not clear. The present study showed that the mRNA expression of Ephx2 and Polr2k decreased after AP treatment, and pre-administration of CGA reversed such decrease. Those results indicate the potential involvement of Ephx2 and Polr2k in the CGA protection from AP-induced liver injury.

Fmo is a superfamily of xenobiotic metabolizing enzymes. There are five members of Fmo in humans, of which Fmo5 is highly expressed in the adult liver (Phillips et al., 1995; Zhang and Cashman, 2006). In general, the CYP family is the major contributor to the metabolism of xenobiotic compounds in the liver. However, Fmo may be of significance in a number of cases and should not be overlooked. We found that AP decreased Fmo5 mRNA expression, but CGA failed to increase it. MT is a family of cysteine-rich, low-molecular-weight proteins, and it has been reported to protect against oxidative stress injury induced by exogenous agents (Bauman et al., 1991; Liu et al., 2009). In addition, there is a report that MT-null mice were more susceptible than wild mice to AP-induced hepatotoxicity, whereas zinc pretreat-

ment, a method of inducing MT expression, protected against AP-induced hepatotoxicity (Liu et al., 1999). Our present study showed that AP induced MT1 and MT2 mRNA expression, which will contribute to the self-protection of the body against AP-induced liver injury (Saito et al., 2010). However, CGA had no effect on the AP-induced increased mRNA expression of MT1 and MT2. SOD is one of the most important antioxidant enzymes in the body's antioxidant systems, which contains SOD1, SOD2, and SOD3 (McCord and Fridovich, 1969). Our previous study showed that ferulic acid prevented diosbulbin B-induced liver oxidative injury by elevating the decreased mRNA expression of SOD1 (Wang et al., 2014), and thus we suspect that SOD1 and SOD2 may contribute to the detoxification of CGA against AP-induced liver oxidative injury. However, our study showed CGA had no effect on the mRNA expression of SOD1 and SOD2. All these results indicate that Fmo5, MT1, MT2, SOD1, and SOD2 may not contribute to the protection of CGA against AP-induced liver injury.

Nrf2, has emerged as a transcription factor, which is capable of inducing a large variety of antioxidant and detoxification enzymes (Klaassen and Reisman, 2010). Prdx1, Sod1, and Sod2 are all reported to be the target genes regulated by Nrf2 (Kim et al., 2007; Reszka et al., 2015). Previous study showed that the mRNA expression of Nrf2 and Prdx1 were both elevated by quercetin treatment, and thus prevented carbon tetrachloride-induced liver oxidative injury (Zhang et al., 2014). Our results demonstrated that CGA reversed the decreased Nrf2 mRNA expression induced by AP. The results indicate that Nrf2 also contributes to the prevention of CGA against AP-induced liver injury.

In summary, our study demonstrated that CGA ameliorates the AP-induced liver injury only slightly through inhibiting its metabolism. In addition, except GSH and Trx antioxidant systems, some other antioxidant signals such as Prx1, 2, 3, 5, 6, Ephx2, and Nrf2 all contributed to the protection of CGA against AP-induced liver oxidative injury.

Compliance with ethics guidelines

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中文概要

题　目：**CYP450 代谢酶和一些抗氧化信号参与调控了绿原酸抑制对乙酰氨基酚的肝毒性**

目　的：本研究旨在观察绿原酸对乙酰氨基酚诱导肝损伤的解毒作用及其机理。

创新点：发现 CYP450 代谢酶和一些重要的抗氧化信号分子（如 Prx 家族蛋白等）参与调控了绿原酸抑制对乙酰氨基酚的肝毒性。

方　法：检测小鼠血清转氨酶含量，检测体外 CYP2E1、CYP3A4 和 CYP1A2 酶活性，检测肝组织中丙二醛（MDA）、谷胱甘肽（GSH）和活性氧（ROS）含量，用实时聚合酶链反应（real-time PCR）检测肝组织中 Prx1-6、Ephx2、Polr2k、Fmo5、Nrf2 等的 mRNA 表达情况。

结　论：绿原酸可以明显抑制对乙酰氨基酚造成的急性肝损伤。给药组小鼠血清中的转氨酶与模型组相比均有显著下降，绿原酸在体外可以微弱抑制 CYP2E1 和 CYP1A2 代谢酶的活性，通过 MDA、GSH 和 ROS 分析发现绿原酸可以抑制对乙酰氨基酚造成的氧化应激损伤。通过 real-time PCR 分析发现对乙酰氨基酚降低了抗氧化酶 Prx 家族、Ephx2、Polr2k 和 Nrf2 的基因表达，而绿原酸可以逆转对乙酰氨基酚降低的这些基因的表达。

关键词：绿原酸；对乙酰氨基酚；CYP450；氧应激损伤